

Green coffee as a modified agent for improving the erosive potentiality of the energy drinks on hard tooth structure

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Received: 17 March 2022

Revised: 8 May 2022

Accepted: 23 May 2022

Published: 24 December 2022

Journal of The Arab Society for Medical Research 2022, 17:118–124

Background/aim

The interest in herbal products has increased in the last years because of their limited adverse effects. The aim of this study was to evaluate the remineralization effect of green coffee on demineralized enamel in comparison with green tea.

Materials and methods

A total of 42 crowns of primary molars were divided equally into buccal and lingual segments (84 totals). Each specimen was embedded into an acrylic block and then immersed in Red Bull for 4h. The specimens were divided into two groups according to the type of remineralizing solution used: group 1 using green tea and group 2 using green coffee. In each group, the buccal halves were subjected to microhardness test, whereas the lingual halves were subjected to scanning electron microscopy and energy-dispersive radiograph analysis examinations.

Results

The results of scanning electron microscopy revealed a demineralization effect after immersion into Red Bull, which decreased after immersion into both remineralizing solutions of green tea and coffee. The energy-dispersive radiograph analysis showed a decrease in calcium and phosphorous weight% after demineralization, which is increased after remineralization with both solutions. The microhardness test revealed a decrease in surface hardness after demineralization, which increased after the remineralization process. Moreover, in comparing the two remineralizing solutions, the remineralization effect of green coffee was statistically higher than that of green tea.

Conclusion

Green coffee has an efficient remineralizing effect than green tea, which was superior in treatment of initial caries.

Keywords:

green coffee, green tea, primary teeth, Red Bull, scanning electron microscope

J Arab Soc Med Res 17:118–124

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1687-4293

Introduction

Dental caries is the most common chronic disease affecting children. Its prevalence reaches from 60 to 90% among school age children [1]. In the first stage of caries formation, dissolution of minerals starts, and after a while, enamel becomes more porous with disruption of its natural architecture. Fortunately, the demineralized lesions that form are reversible if a suitable environment is created. This environment may be accomplished by remineralization therapies that aim to regain the lost mineral content into the demineralized pores by precipitating calcium and phosphate either from external sources or from those available already in saliva [2].

Acidic foods and drinks are the most common cause of tooth erosion. One of these drinks is the energy drink Red Bull, which has a high erosive potential on human teeth, owing to its high sugar content and low pH, which cause destructive effects on the enamel surface [3,4].

Recently, the introduction of herbal products has gained popularity owing to their beneficial properties with no side effects. Green tea and green coffee are among the herbal products that have received greater attention owing to their antimicrobial effects against gram-negative and gram-positive bacteria as they showed a statistically significant reduction in *Streptococcus mutans* count in previous studies [5].

Moreover, green tea has the ability of protecting the eroded enamel by increasing the surface microhardness and decreasing the surface roughness of the eroded enamel. Green tea also is known to contain polyphenols/catechins in large amounts, which makes the green tea extract exhibits true functional remineralization [6,7].

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The aim of this study was to evaluate the remineralizing effect of green coffee on initial caries-like lesions produced by Red Bull energy drinks in comparison with green tea.

Materials and methods

Materials

Red Bull: Red Bull (Red Bull Srl, Milano, Italy) was purchased from market as 250-ml disposable cans.

Green tea: 100% green tea produced by Ahmed Tea FZ - LLC, Ras Al Khimah, United Arab Emirates, and purchased from a market in Cairo, Egypt, was used.

Green coffee: 100% Green Coffee Arabica, Abu Zeid Coffee, Egypt, purchased from a local market in Cairo was used.

Teeth collection

In the current study, 42 primary molars were freshly extracted due to normal shedding or for orthodontic purposes from 6 to 10-year-old children and were collected from the Pedodontics Clinic, Nahda University, Beni-Suef, Egypt.

The teeth were cleaned off any soft tissues and inspected for the presence of any cracks, hypoplasia,

or other clinical defects. Only teeth with an intact enamel surface were included in this study. Samples were then kept in a 0.10% thymol solution until the time of use.

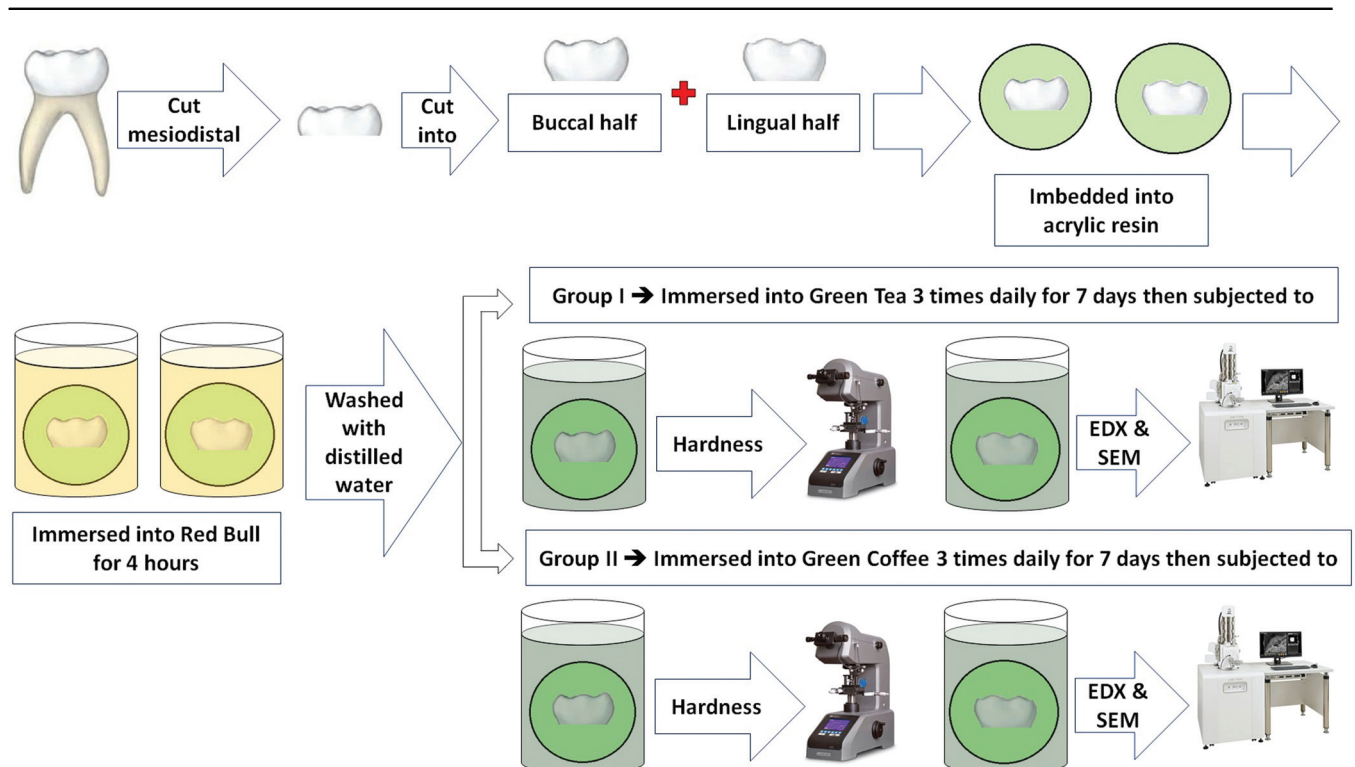
The specimens were sectioned mesio-distally from the cervical area to separate the crown from the root. Each crown was further sectioned into two halves (buccal and lingual) to obtain 84 specimens. All the specimens were then embedded in an acrylic resin block. The buccal halves were subjected to Microhardness test, whereas the lingual halves were subjected to scanning electron microscopy (SEM) and energy-dispersive radiograph (EDX) examinations, as illustrated in Fig. 1.

Study design

The representative scheme in Fig. 1 shows a summary of the methodology of the current study. However, all of the specimens of teeth were immersed in Red Bull for 4 h, and then washed with distilled water to remove any remnants of the demineralizing solution [8]. Specimens were randomly distributed into two groups (42 each).

Group 1: the specimens were immersed in a beaker filled with a freshly prepared green tea solution for 5 min, three times daily for 7 days, to simulate a

Figure 1



Representative scheme showing the methodology of the current study.

discontinuous but prolonged drink assumption [9]. After the immersion protocol, the specimens were washed and stored in distilled water.

Group 2: the specimens were immersed in a beaker filled with a freshly prepared green coffee solution with the same protocol as in group 1 and then washed and stored in distilled water.

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. This study has been approved by the local Ethics Committee of National Research Centre, Cairo, Egypt, with approval number 14/350. All parents after having a written and verbal explanation of the study signed a consent form to clarify their approval for participation in this study.

Methods

Preparation of immersing solutions

- (1) Red Bull: the solution was degassed by stirring for 10 min at room temperature.
- (2) Green tea: a tea bag (2 g) was placed in 100 ml of boiled distilled water and kept for 5 min. The solution was then left to cool until reaching room temperature.
- (3) Green coffee: 2 g of green coffee was added to 100 ml of water, then boiled for 10 min, and then filtered using a gauze. The solution was left to cool until reaching room temperature.

Scanning electron microscopy

The specimens of all groups, before and after remineralization, were mounted on stubs without coating and examined using a Scanning Electron Microscope Unit (JSM-IT200 Jeol, Jeol Ltd. Massachusetts, USA) for determination of the morphological changes occurring on the tooth surface. Specimens were mounted on the SEM plate, and the surface enamel was examined at different magnifications by the same operator.

Energy-dispersive radiograph analysis

The quantitative composition of the studied surfaces was determined by EDX, which measured the number of emitted radiograph versus their energy. This analysis system works as integrated features of SEM (JSM-IT200, Jeol). Data were represented as wt.% for calcium, phosphorus and carbon. This analysis was done at three points: baseline, after demineralization, and after remineralization in both groups.

Microhardness test

Surface microhardness of the buccal specimens at the same points as in the EDX test was measured by Vickers hardness tester (Tukon 1102 Wilson microhardness tester Buehler Germany). Three indentations were made with a rate of 10 s and a load of 100 gf (HV 0.1) at a distance of 500 µm from the edge, and three readings were recorded for each specimen.

Sample size calculation

For calculation of sample size, G power, version 3.1.9.7 (Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany) was used. It was calculated with a power of 95% and a significance level of 95% according to a one-way analysis of variance study [10]. The minimum sample size was calculated to be 32 sound molar (16 for each group) teeth. This was increased to 42 (21 for each group) (30% increase) to make up for errors in processing [11].

Statistical analysis

Data were analyzed with IBM SPSS Statistics (Armonk, New York, USA), version 25 for Windows. For each group, the mean and SD values were calculated. Normality test was done using Kolmogorov-Smirnov test and clarified normal distribution between values of each group. Homogeneity test was done using Levene's test and revealed that there was a homogenous distribution between all variables. Therefore, one-way analysis of variance, Tukey post-hoc, and independent *t* tests were accomplished (with significance level set at $P \leq 0.05$) to reveal the statistically significant difference between the variables.

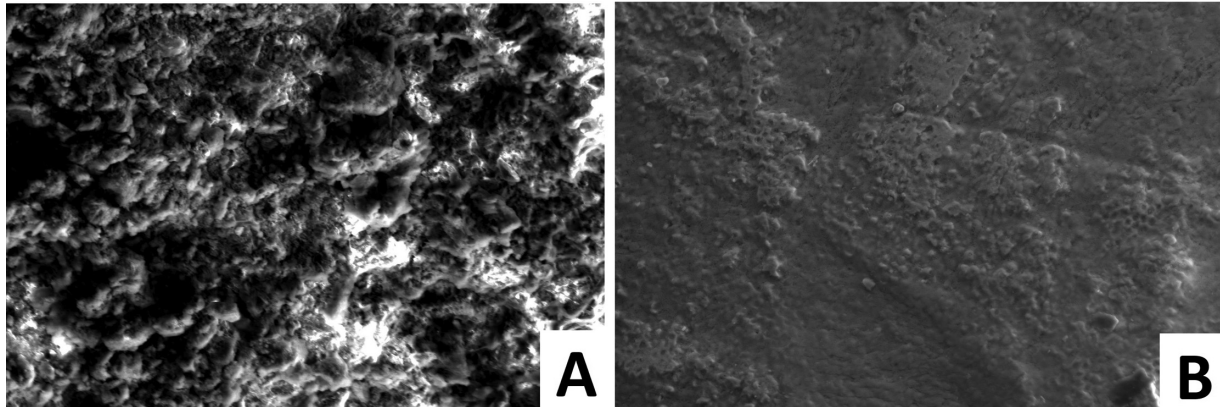
Results

Scanning electron microscopy results

After demineralization, SEM images revealed a loss of surface integrity resembling enamel erosive lesions. The surface was irregular, associated by a number of porous defects (the structure appeared as dark areas), with a honeycomb appearance of demineralization, which is a peculiar characteristic of carious enamel (Fig 2a and Fig. 3a).

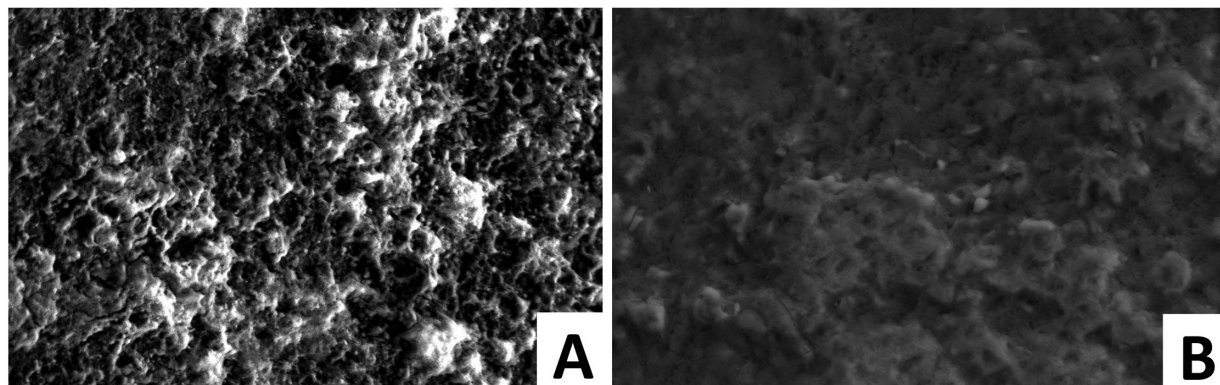
After immersion into green tea (Fig. 2b) and green coffee (Fig. 3b), SEM images showed an important feature in both groups in which there was a relatively decrease in the porosities and surface irregularities. This may be revealed by regenerated crystals, which formed a homogeneous and dense layer of mineralized tissue in some areas of the enamel surface that revealed a process of remineralization.

Figure 2



Scanning electron micrographs of green tea samples (group 1); (a) after immersion into Red Bull, (b) after immersion into green tea.

Figure 3



Scanning electron micrographs of green coffee samples (group 2) samples; (a) after immersion into Red Bull, (b) after immersion into green coffee.

Table 1 Calcium, phosphorous, carbon and calcium/phosphorus weight% by energy-dispersive radiograph at the three measuring stages for both groups of green tea (group 1) and green coffee (group 2)

	Calcium	Phosphorous	Carbon	Ca/P
Group 1 (green tea)				
Baseline	29.59±2.06 ^a	15.91±0.92 ^a	8.08±0.71 ^b	1.86±0.04 ^a
Demineralization (Red Bull)	24.51±2.51 ^b	13.29±0.94 ^b	11.25±1.06 ^a	1.66±0.04 ^b
Remineralization (green tea)	30.12±2.37 ^a	15.11±0.46 ^a	8.98±0.49 ^b	1.99±0.15 ^a
Group 2 (green coffee)				
Baseline	29.90±1.99 ^a	16.52±0.67 ^a	8.11±0.93 ^b	1.88±0.06 ^a
Demineralization (Red Bull)	24.03±0.61 ^b	13.60±0.48 ^b	11.31±1.05 ^a	1.72±0.06 ^b
Remineralization (green coffee)	32.35±2.07 ^a	16.06±0.45 ^a	9.18±0.67 ^b	1.89±0.04 ^a

All data are expressed as mean±SD. Results with different letters (a, b, c) within the same column are significantly different at *P* value less than or equal to 0.05, using analysis of variance test.

Energy-dispersive radiograph

The weight percent of surface elements before and after immersion into solutions is listed in Table 1. The weight percent values at baseline and after remineralization with both solutions of calcium, phosphorous, carbon, and calcium/phosphorous ratio showed insignificant difference.

However, these values showed statistically significant difference with demineralization with Red Bull values.

Hardness

The hardness values are tabulated in Table 2. The highest hardness value was recorded at baseline in both

Table 2 Hardness (VHN) at the three measuring stages for both groups of green tea and green coffee

Time	Group 1 (green tea)	Group 2 (green coffee)
Baseline	278.22±10.7 ^a	307.97±5.0 ^a
Demineralization	216.87±10.77 ^b	231.18±13.2 ^b
Remineralization	253.77±13.2 ^c	275.03±11.5 ^c

All data are expressed as mean±SD. Results with different letters (a, b, c) within the same column are significantly different at *P* value less than or equal to 0.05, using analysis of variance test.

groups (278.22±10.7 for tea group and 307.97±5.0 for coffee group).

The immersion into demineralizing solution (Red Bull) significantly decreased the surface hardness in both groups (216.87±10.77 for tea group and 231.18±13.2 for coffee group). However, immersion into both remineralizing solutions recorded a statistically significant increase in the hardness values in both groups: 253.77±13.2 for group 1 (green tea) and 275.03±11.5 for group 2 (green coffee). However, surface hardness values after immersion into both remineralizing solutions were statistically significantly lower than those recorded at baseline.

The remineralizing effect of green tea and green coffee over the demineralized enamel surface was calculated by subtracting the demineralization values from the remineralization values (Table 3). The values of calcium, phosphorus, calcium/phosphorous ratio weight percent, and hardness of group 2 (green coffee) showed a higher statistically significant difference than values of group 1 (green tea). However, values of carbon weight percent showed insignificant difference between both groups.

Discussion

The caries process still remains a major public health problem. Despite the enamel remineralizing capacity of saliva, the process of increasing calcium and phosphate levels cannot be initiated by itself. Remineralization was a major investigative area for preventing the beginning and disruption of enamel erosion [12]. Thus, this study aimed to determine the remineralizing effect of green coffee on initial caries-like lesions produced by Red Bull energy drinks compared with green tea.

Energy drinks are soft drinks with the addition of some vitamins that increase the concentration and stimulate body metabolism, thus give more energy to the consumer. Unfortunately, the consumption of energy drinks among young children has remarkably increased. Therefore, many researches are enforced

Table 3 The positive effect of remineralizing solutions on the values of calcium, phosphorous, carbon, calcium/phosphorous (weight %) and hardness of both groups of tea (group 1) and coffee (group 2)

	Group 1 (green tea)	Group 2 (green coffee)	<i>P</i> value
Calcium	6.11±1.00	8.98±1.29	0.002*
Phosphorus	1.48±0.30	3.30±0.39	0.000*
Carbon	1.60±0.28	1.55±0.47	0.817
Ca/P	0.18±0.05	0.30±0.04	0.002*
Hardness	39.90±3.65	43.85±6.32	0.042*

All data are expressed as mean±SD. *Significant difference between two groups at *P* value less than 0.05, using *t* test.

to assess the erosive potential of energy drinks, as the acidity of food and beverage products is one of the main factors related to tooth erosion [4,13].

Owing to the fact that children were the dominant consumers of energy drinks, primary extracted teeth were chosen as tested participants in the current study. However, enamel of primary teeth is less mineralized owing to increased organic content, greater diffusion coefficient, and consequently high susceptibility to acid dissolution compared with enamel of permanent teeth resulting in weaker mechanical properties. Accordingly, the effect of these energy drinks may become more exaggerated in children [14,15].

Regarding energy drink consumption, the exposure to Red Bull in this study caused enamel demineralization; EDX results presented statistically significant decrease in Ca and P weight percent after immersion into Red Bull as a result of increased destruction of hydroxyapatite crystals. These data were confirmed by SEM, which showed a more damaging effect of Red Bull to enamel surface. This result was in agreement with Owens [16], who explained that Red Bull had high titratable acidity, which increased the erosive potentiality of the enamel matrix.

Natural products have been the focus of many research studies in the last years to be used as new therapeutic agents for remineralization. Green tea and coffee are natural products that are recently used for various therapeutic purposes. Green tea was selected to be compared with the tested green coffee because of its antibacterial activity through inhibition of salivary amylase activities, glucosyltransferase, and bacterial growth. The high content of polyphenols/catechins like epigallocatechin gallate in green tea makes it effective in the remineralization process [7,17].

The results of the present study showed that both drinks showed a significant positive remineralization

effect against the erosive potentiality of acidic drinks. Unfortunately, this remineralizing potentiality in both groups did not approach the original values of a sound tooth. This may be attributed to the enamel crystal formed after remineralization in both groups. Hydroxyapatite crystals, which were formed after application of green tea and coffee, are amorphous molecules. This amorphous form has an irregular structure which decrease the density of the crystals and allows for its easier decomposition when subjected to external forces [18].

When comparing the two remineralizing solutions, green coffee showed a significant higher remineralization effect on demineralized enamel when compared with green tea. This may be referred to as the difference in the remineralization mechanism of both solutions. Green coffee in this study produced a remineralizing effect on the demineralized enamel surface which was proved by the increase in the concentration of calcium and phosphorous and the hardness value of the demineralized enamel. However, the reason for the significant increase in hardness and the relatively remineralized effect in SEM for the green coffee over the green tea group in this study may be attributed to the presence of chlorogenic acid and polyphenol, which are considered the main constituents of green coffee. The level of chlorogenic acid (the active ingredient) is high in green coffee when compared with the roasted form of coffee [5]. Zhang *et al.* [19] stated that the reaction between the polyphenols and the organic matrix of enamel leads to inhibition of the demineralization process. This interaction involves different bonds such as covalent, ionic, and hydrogen bonding, which results in alteration of the enamel organic matrix. This altered organic matrix can be precipitated in the structure of enamel leading to reduction in mineral ions loss and inhibition of enamel demineralization. In addition, Kashket *et al.* [20] found that polyphenols inhibit the formation of glucosyltransferase by cariogenic bacteria. Moreover, Qasim *et al.* [21] reported that the presence of chlorogenic acid in coffee limited the erosive effect of the ascorbic acid on enamel surface roughness.

On the contrary, green tea also promoted enamel remineralization through the percent changes in microhardness and this had been confirmed by the SEM examination. In addition, the analysis of EDX of the green tea in this study showed significant increase in the calcium and phosphorus content after application on demineralized enamel. This result was in agreement with previous studies, which examined microscopical changes of different types of tea in

preventing tooth decay and revealed that green tea produced adequate enamel remineralization [22,23]. The lower remineralization potentiality of green tea when compared with green coffee may be attributed to the fact that green tea contains fluoride in small amounts, which helps in precipitation of calcium phosphate on the demineralized enamel and formation of fluor-hydroxyapatite crystals, which is different in form and density than that formed in green coffee [24,25].

Furthermore, the concentration of carbon content was increased after the application of demineralized solution and then it decreased after remineralization in contrast to calcium and phosphorous. This may be explained as the carbon may substitute the phosphorous in the hydroxyapatite crystals, leading to decrease in its level [26,27].

Conclusion

From the previously mentioned results, green coffee has some advantages over green tea as it causes significant increase in the remineralization process. Moreover, it can be considered as a good preventive home therapy for initial spot caries in developing countries as it adds a great health benefit over the human body. Within the limitations of the current study, it can be concluded that both green tea and green coffee produced a remineralization effect on demineralized enamel. However, green coffee produced a greater remineralization effect than green tea, but regrettably, they did not regain the normal tooth structure of a sound tooth.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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