The influence of topical application of grapeseed extract gel on enamel surface hardness after demineralization

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Abstract. The aim of this study was to analyze the influence of topical application of 6.5% and 12.5% grapeseed extract gels for duration of application 16 and 32 minutes on the enamel surface hardness following tooth demineralization by an energy drink. The samples were 21 bovine teeth that underwent demineralization by immersion in the energy drink for 5 minutes in an incubator at 37°C. The demineralized specimens were randomly divided into a control group and 2 treatment groups. The control group was immersed in artificial saliva for 6 hours at 37°C, whereas the treatment groups were treated with topical 6.5% and 12.5% grapeseed extract gels for durations of 16 and 32 minutes and then immersed in artificial saliva for 6 hours at 37°C. The hardness was measured with a Knoop hardness tester. Statistical analysis by repeated ANOVA and one-way ANOVA revealed a significant increase in the enamel hardness value \((p<0.05)\) after the application of the topical grapeseed extract gels at both concentrations. Application of 12.5% topical grapeseed extract gel for 32 minutes resulted in a restored hardness that insignificant difference from the initial hardness value obtained before demineralization \((p>0.05)\).

1. Introduction

Human teeth consist of 4% organic components and 96% inorganic components, such as calcium hydroxyapatite and other minerals, as well as collagen and water [1]. Every day, teeth have demineralization and remineralization cycle. Demineralization is a mineral loss process that causes a hardness reduction in the enamel and dentine surface [2,3], which caused tooth caries [4]. Demineralization is influenced by food and drink consumption. High acidic foods consumptions will increase the oral acidity level and promote tooth demineralization. One example of a highly acidic drink is the energy drink [3], a dietary supplement, with or without caffeine, that contains multivitamins, macronutrients (carbs and protein), and taurine; ginseng and ginger are usually added. These kinds of drinks are consumed to increase energy and refreshment, stimulate metabolism, and maintain health and stamina, so they are often consumed while working or after exercising [5].

The high consumption of energy drinks can lead to tooth erosion [3]. Malinauskas et al. showed that energy drink consumption levels were high in university students, 51% of them routinely drinking energy drinks to improve their ability to exercise and function in daily life [6]. Rabobank’s research in 2011 indicated that energy drink sales growth will increase by 15% in the next 5 years in Indonesia, confirming a high level of energy drink consumption. This consumption was influenced by a city lifestyle and daily business [7]. Energy drinks have a low pH, which can cause tooth erosion. However, the acidity level alone is not sufficient to predict the erosive potential of energy drinks. The
erosive potential is further influenced by the low degree of hydroxyapatite and fluorapatite saturation and the presence of cirate, a substance that can eliminate calcium from saliva and teeth [8].

Demineralization can be prevented by restoring tooth mineral components through the process known as remineralization. Common remineralization agents include fluoride, casein-phosphopeptide–amorphous calcium phosphate (CPP-ACP), and beta-tricalcium phosphate (β-TCP) proven to prevent caries [2,9,10]. Remineralization can also be achieved with natural ingredients that have been used for a long time as medications. One example is grapeseed extract, a supplement that is often used as an antioxidant and is known to contain proanthocyanidin (PA); this extract is an effective remineralization agent [11]. In 2008, Qian Xie et al. showed that application of a 6.5% grapeseed extract could contribute in remineralization of dentin in permanent tooth roots [12]. Research conducted by Mahkameh et al. in 2013 also showed that a 12.5% grapeseed extract had a positive effect on the remineralization process of primary tooth enamel surfaces [13]. Grapeseed extract applied to the enamel surface acted as a remineralization agent by depositing minerals onto lesion surfaces by forming an insoluble complex when mixed with phosphate buffer [13]. The aim of the present study was to investigate the influence of topical grapeseed extract gels (6.5% and 12.5% concentration), applied for durations of 16 and 32 minutes, on the enamel surface hardness in teeth demineralized by an energy drink.

2. Materials and Methods

This research was laboratory experimental research conducted in the Material Research and Development Laboratory of the Faculty of Dentistry Universitas Indonesia, and The Material Laboratory of The Mechanical and Aerospace Engineering of Institut Teknologi Bandung. Application durations of 16 and 32 minutes were considered as analogous to applications of grapeseed extract topical gel for 1 and 2 months with the calculation of once application is 4 minutes per week. [2]. The research was designed to investigate the influence of topical grapeseed extract gels as an alternative agent for tooth remineralization, to improve enamel surface hardness after demineralization by an energy drink. The tooth enamel used in this research was bovine tooth enamel, because this enamel has almost the same structure and composition as human tooth enamel [14].

Twenty tooth enamel specimens were cut at the cemento-enamel junction to separate the crown and root. The crown parts were cut to obtain one third of the middle and cut into 5mm×5mm squares. The tooth crown specimens were then embedded in decorative acrylic resin, finished using SiC number 2000, and polished using a water-based 1µm alumina suspension. The initial enamel hardness value was recorded using a microhardness tester (Knoop Zwick Roell Microhardness Tester, Zhµ) with a weight of 50g/15secs [15]. Five indentations were made on each specimen and the mean values were calculated. The specimens were then immersed in the energy drink (Kratingdaeng®) for 5 minutes in an incubator at 37ºC to create lesions in the specimen surface [16]. The specimen hardness values were then recorded as previously.

The specimens were randomly divided into 3 groups: a control group and 2 treatment groups, each containing 7 specimens. The control group was immersed in artificial saliva (NaCl, KSCN, NaHCO3, KCl, Urea, Na2HPO4, KH2PO4, and distilled water, pH 7) for 6 hours in an incubator at37ºC for a remineralization process designed to mimic oral remineralization. The two treatment groups were treated with 6.5% and 12.5% grapeseed extract topical gel for durations of 16 and 32 minutes before immersion in artificial saliva for 6 hours at 37ºC for remineralization. Following the remineralization process, the specimen hardness values were determined as previously. The mean enamel hardness value was then analyzed using a repeated ANOVA test to determine the statistical significance of the observed differences in demineralized enamel hardness values following energy drink exposure and after application of grapeseed extract topical gel. One-way ANOVA comparative tests were also done to determine the differences in the enamel hardness values among the 3 groups.
3. Results and Discussion

3.1 Results

Table 1 shows the mean of enamel hardness values before and after demineralization by the energy drink and after immersed in artificial saliva. Also shown the mean of enamel hardness value after application of 6.5% and 12.5% grapeseed extract topical gel for 16 and 32 minutes.

Table 1. Mean values of Enamel hardness (KHN)

|                         | Control (artificial saliva) | 6.5% Grapeseed extract | 12.5% Grapeseed extract |
|-------------------------|-----------------------------|------------------------|-------------------------|
| Before                  | 427±3                       | 427±3                  | 427±3                   |
| Demineralization        | 294±3                       | 294±3                  | 294±3                   |
| Artificial saliva       | 327±1                       |                        |                         |
| 16 minutes' application + Artificial saliva | 363±2                       | 388±1                  |                         |
| 32 minutes' application + Artificial saliva | 386±1                       | 424±1                  |                         |

The results in table 1 show a decrease in the enamel hardness value after immersed in the energy drink, and an increase in enamel hardness value after remineralization, either following artificial saliva treatment or following application of the grapeseed extract topical gel and subsequent artificial saliva treatment. All 3 groups showed significant decreases in enamel hardness values after demineralized by energy drink ($p<0.05$), the enamel hardness values decreased from 427±3 KHN to 294±3 KHN (31% decrease). The enamel hardness values of the teeth demineralized by the energy drink were significantly increased after remineralization in artificial saliva ($p<0.05$), from 294±3 KHN to 327±1 KHN (11% increase). The group treated with 6.5% topical grapeseed extract gel for 16 also showed a significant increase in the enamel hardness value when compared to the enamel hardness after demineralization ($p<0.05$), from 294±3 KHN to 363±3 KHN (23% increase). The enamel hardness value for the group treated with 6.5% topical grapeseed extract gel for 32 minutes was significantly increased when compared to the enamel hardness after demineralization ($p<0.05$), from 294±3 KHN to 388±1 KHN (31% increase). The enamel hardness value after 32 minutes of application was significantly increased compared to the teeth applied for 16 minute duration of application, from 363±3 KHN to 388±1 KHN (6% increase).

The group treated with 12.5% topical grapeseed extract gel for 16 minutes showed a significant increase in the enamel hardness value when compared to the enamel hardness after demineralization ($p<0.05$) (from 294±3 KHN to 366±1 KHN; 32% increase). The group treated with 12.5% topical grapeseed extract gel for 32 minutes showed a significant increase in the enamel hardness value compared to the enamel hardness after demineralization ($p<0.05$), from 294±3 KHN to 424±1 KHN (44% increase). The enamel treated with 12.5% topical grapeseed extract for 32 minutes restored the enamel hardness almost reaching the initial hardness value of 427±3 KHN. The enamel hardness value after 32 minutes of application was not differ significantly compared to the initial hardness value. The enamel hardness value after 32 minutes of application was significantly increased when compared to the value after 16 minutes of application, from 363±3 KHN to 388±1 KHN (6% increase).

The groups treated with topical grapeseed extract gel for 16 minutes showed a significant increase in the enamel hardness value when the group treated with 6.5% grapeseed extract was compared with the group treated with 12% grapeseed extract. The hardness values significantly increased after treatment with 6.5% and 12.5% grapeseed extract topical gel when compared to the control group ($p<0.05$). A similar finding was observed for the group treated with topical grapeseed extract gel for 32 minutes, the enamel hardness value was significantly higher in the group treated with 12.5% grapeseed extract than in the group treated with 6.5% grapeseed extract. Significant hardness value increases were observed after application of 6.5% or 12.5% grapeseed extract topical gel, when compared to the control group ($p<0.05$).
3.2 Discussion

This study showed that the enamel hardness value decreased by 31% following immersion of teeth in an energy drink due to the acidogenic properties of these drinks, which show a pH range from 3–4. This value is below the critical pH of hydroxyapatite of 5.5; therefore, demineralization occurs [2,17]. Demineralization is a mineral loss process in teeth, leads to enamel porosity, which decreases the enamel microhardness value [4]. Energy drinks can soften the enamel in just 5 minutes, because the citrate in energy drinks acts as a chelator agent that has the ability to eliminate calcium from the saliva and teeth [3]. In the present study, following demineralization in the energy drink, the control group teeth were immersed in artificial saliva for 6 hours for remineralization under conditions mimic the oral cavity remineralization process. The increase in enamel hardness value as much as 11% after demineralization occurred because remineralization process. Remineralization, which is the process of restoring new minerals onto demineralized teeth, occurs if the pH return neutral and there are adequate calcium and phosphate ions in the saliva. Artificial saliva is designed to have a neutral pH and contains calcium and phosphate ions for remineralization. However, remineralization with artificial saliva alone is not sufficient to return the enamel hardness to its initial value. A remineralizing agent is needed to improve remineralization on enamel teeth, and topical grape seed extract serves this purpose.

Specimens in the second group were treated with 6.5% grapeseed extract topical gel for 16 and 32 minutes. The hardness value following demineralization increased by 23% after the 16 minute application and increased 31% after 32 minutes application. The enamel hardness value was significantly increased after topical application of grapeseed extract gel when compared to the control teeth that were only immersed in artificial saliva, because the grapeseed extract gel could increase mineral deposition onto the tooth enamel [11,12]. In addition, grapeseed extract can also facilitate collagen crosslinking [18]. Felzeghy et al. showed that type X collagen present on the enamel matrix produced positive effects on remineralization by collagen crosslinking [19]. During the mineralization process, collagen peptides absorb onto the hydroxyapatite surface. The presence of these peptides on the hydroxyapatite surface then increases calcium and phosphate absorption, thereby inducing hydroxyapatite crystal growth [13,19].

The repeated ANOVA statistical test conducted on the group treated with 6.5% grapeseed extract topical gel revealed a statistically significant hardness increase when compared to the hardness after demineralization. Immersion in artificial saliva alone greatly increased the hardness value, but application of 6.5% grapeseed extract topical gel for 16 and 32 minutes could improve the hardness better. The enamel hardness value was higher and statistically significant following application of 6.5% grapeseed extract topical gel for 32 minutes than for 16 minutes, indicating an effect of the duration of application on the enamel hardness value, where a longer application duration of the grapeseed extract topical gel gave a higher enamel hardness value. The enamel hardness value after application of 6.5% grapeseed extract topical gel for 16 and 32 minutes was significantly lower than the initial enamel hardness value. Therefore, the application of 6.5% grapeseed extract topical gel for 1 or 2 months would not be expected to restore the enamel microhardness value to the initial value.

The third group, which was treated with 12.5% grapeseed extract topical gel before immersion in artificial saliva, showed an increase of 31% in the enamel hardness value after a 16 minute treatment, when compared to the hardness after demineralization. After 32 minutes, the enamel hardness value was 44% higher than the hardness value after demineralization. The repeated ANOVA statistical test on the group treated with 12.5% grapeseed extract topical gel revealed that this hardness value increase, when compared to the hardness value after demineralization, was statistically significant. The enamel hardness value was higher and more significant following application of 12.5% grapeseed extract topical gel for 32 minutes than 16 minutes. The repeated ANOVA statistical test also showed no significant difference between the enamel hardness value following application of 12.5% grapeseed extract topical gel for 32 minutes and the initial hardness value before demineralization. Therefore, application of a 12.5% grapeseed extract topical gel for 2 months would be expected to restore the enamel microhardness value to its initial value before the demineralization occurred.
One-way ANOVA tests performed on the three groups revealed significant differences between the control group and the groups treated with 6.5% and 12.5% grapeseed extract topical gel. The grapeseed extract topical gel concentration therefore influenced the enamel hardness value, so that a higher concentration of the gel gave a higher increase in the enamel hardness value. These findings confirm that the increases in enamel hardness value are influenced by the concentration of the grapeseed extract topical gel and the duration of the application. An increase in the gel concentration would be expected to shorten the required application time.

4. Conclusion
The findings from this research indicate that grapeseed extract topical gel application for 1 or 2 months could restore the enamel microhardness value after demineralization caused by energy drinks. Application of a 12.5% grapeseed extract topical gel for 2 months could be particularly effective at this restoration. Overall, a higher concentration of grapeseed extract topical gel and a longer duration would appear to provide a larger increase in the enamel microhardness value.

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