World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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The use of white pomegranate extract as a bleaching material for hardness, roughness, and calcium levels in the enamel tooth

Ige Frameski Radila Muga [1,*], Asti Meizarini [2], Intan Nirwana [2], Moh. Khafid [3], Basma Rosandi Prakosa [4] and Dzanuar Rahmawan [5]

[1] *Department of Dental Material, Faculty of Dentistry, Institut Ilmu Kesehatan Bhakti Wiyata, Kediri, East Java, Indonesia.*
[2] *Department of Dental Material, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, East Java, Indonesia.*
[3] *Department of Dental Public Health, Faculty of Dentistry, Institut Ilmu Kesehatan Bhakti Wiyata, Kediri, East Java, Indonesia.*
[4] *Department of Odontology Forensic, Faculty of Dentistry, Institut Ilmu Kesehatan Bhakti Wiyata, Kediri, East Java, Indonesia.*
[5] *Department of Biomedic, Faculty of Dentistry, Institut Ilmu Kesehatan Bhakti Wiyata, Kediri, East Java, Indonesia*

World Journal of Advanced Research and Reviews, 2023, 18(02), 700–704

Publication history: Received on 05 April 2023; revised on 14 May 2023; accepted on 16 May 2023

Article DOI: https://doi.org/10.30574/wjarr.2023.18.2.0855

Abstract

Background*:* Bleaching is the procedure of re-whitening teeth that have darkened to a shade near to their natural hue. Due to the unfavorable impact of chemical bleaching materials on enamel demineralization, the quest for natural bleaching solutions that have the same whitening effect while minimizing the negative consequences is encouraged. The punica granatum fruit is one of the natural components that can be employed.

Purpose: This study aims to analyze the application of white pomegranate extract as an external bleach on the physical properties of the enamel surface of the tooth.

Methods: thirty post-extraction bovine teeth were divided into treatment and control groups, then enamel surface hardness was measured using a Micro Vickers Hardness Tester, enamel surface roughness using a Surface Roughness test, and calcium levels using an EDS SEM tool before application of white pomegranate extract which is performed for 2 hours a day for two weeks. After two weeks, the enamel surface hardness, enamel surface roughness, and calcium levels were measured again.

Results: The results of the treatment group using white pomegranate extract showed that there was no decrease in enamel surface hardness ($p>0.05$), a decrease in enamel roughness ($p<0.05$), and no decrease in calcium levels ($p>0.05$). The control group's results using 16% carbamide peroxide showed a decrease in the hardness and surface roughness of the enamel, but it did not decrease the calcium content.

Conclusion: white pomegranate extract can be used as an external bleaching agent because of not reduce the surface hardness, reduce the surface roughness and not reduced the calsium of the enamel tooth

Keywords: *Punica granatum*; Enamel surface hardness; Enamel Surface Roughness; Calcium levels

* Corresponding author: Ige Frameski Radila Muga
Department of Dental Material, Faculty of Dentistry, Institut Ilmu Kesehatan Bhakti Wiyata, Kediri, East Java, Indonesia.

1 Introduction

In the last few decades, the desire for patients to have a smile with white teeth has increased tremendously (1). For most individuals, tooth color is critical, and discoloration or staining of teeth can have a negative impact on their quality of life. Tooth discoloration is caused by intrinsic and extrinsic factors, including smoking, consuming tannin-rich foods, and using chlorhexidine (2). Teeth whitening can be used to address tooth whitening (bleaching). The most often used teeth bleaching chemicals are hydrogen peroxide or carbamide peroxide in gels or toothpaste with variable strengths (3). Hydrogen peroxide bleaching substance has a detrimental effect on dental enamel since it can induce demineralization (4). Demineralization is the process of removing mineral ions from tooth enamel. The main content of enamel is hydroxyapatite (HA) which consists of $Ca_{10}(PO4)_6(OH)_2$. The demineralization process occurs due to the release of calcium (Ca^{2+}) bonds from the phosphate compound hydroxyapatite ($Ca_{10}(PO4)_6(OH)_2$) tooth enamel as a result of changes in the micro texture of tooth enamel (5). The negative effect of using bleaching materials from hydrogen peroxide or carbamide peroxide has led to the desire to look for natural bleaching materials that have the same whitening effect as hydrogen peroxide and minimize the adverse effects. One of the natural ingredients that can be used is pomegranate (*Punica granatum L.*) because it contains ellagic acid.

Pomegranates come in three varieties in Indonesia: white pomegranate, red pomegranate, and black pomegranate. Because white pomegranates contain a higher concentration of flavonoids than other pomegranates, they are frequently employed as herbal medicinal components. Ellagic acid is a powerful oxidizing chemical that acts as a teeth whitener in white pomegranates. Previously research reported that pomegranate (*Punica granatum L.*) could considerably alter discolored teeth' value and chroma ratings (6). However, the status of tooth enamel structure following pomegranate usage as a teeth whitener is unknown. This study aimed to determine the effect of external bleaching using white pomegranate extract on the physical qualities of the enamel tooth surface.

2 Material and methods

This study takes place in actual experimental laboratories and employs a pre-and post-test control group design. The research sample consisted of bovine incisor teeth that were caries-free, fracture-free, and abrasion-free. Thirty pieces were utilized as samples, separated into two groups: 1). White pomegranate extract was employed in the teeth whitening sample group 2). The control group utilized 16 percent carbamide peroxide for tooth whitening. The hardness, surface roughness, and calcium content of each group were determined.

To detach the crown from the root, the cementoenamel junction of the tooth was sliced. With the labial side facing up, the crown of the tooth was implanted in the mold using self-cured acrylic. The white pomegranate extract was prepared by peeling the fruit and separating it from the skin, mashing it with a blender, adding 96 % ethanol solvent, stirring, and leaving the container closed for five days. The container was then filtered using filter paper and a Buchner funnel, and the process was completed by evaporation.

The study procedure began by immersing the tooth sample in a glass of complete human saliva prepared by preserving saliva in a 10 ml bottle for 24 hours. The tooth sample was extracted and rinsed under running water to eliminate any remaining saliva. Tissue paper was used to dry the tooth sample, and then white pomegranate extract was applied to the labial surface of the tooth twice daily for two hours and put in an incubator set at 370C. The sample was washed under running water and dried with tissue paper before being reintroduced into a glass of saliva. Daily, the saliva in the glass is replaced. The technique of immersion lasted two weeks.

The enamel surface hardness was determined prior to and during pomegranate extract application using a Micro Vickers Hardness Tester (Shimadzu HMV-M3 Japan) loaded with 200 gr for 10 seconds. The enamel surface hardness of each sample was determined at three indentation places on the incisal, middle, and cervical thirds of the tooth. The indentation's outcome was seen using a 400x magnification microscope lens, revealing a rhombus shape. The diagonal length is determined by placing two markings at the end of the rhombus formation; the result is the surface hardness (VHN).

The roughness of the enamel surface was determined before and after applying white pomegranate extract. After removing the sample from the saliva bath and patting it dry using tissue paper, it was placed on an iron block, and the teeth were fastened on a Surface Roughness tester (Sufcorder SE 1700 Japan). The tactile needle (stylus) is positioned to correspond to the tooth's labial surface. The stylus can be moved automatically by the driving motor. After starting the measuring equipment, the results from the roughness measurement may be viewed on the tool's monitor screen.

Calcium levels were determined before and following the application of white pomegranate extract. The sample area to be studied on all sample surfaces was determined, followed by scanning using a scanner attached to an SEM-EDS instrument (Hitachi Flexsem 1000 Japan) and obtaining results in less than a second. The SEM-EDS screen displays the collected findings. In all treatment groups of pomegranate extract and 16 percent carbamide peroxide, the Paired Sample t-test or Wilcoxon's test was utilized to analyze the results, depend on the normality distribution and the varians homogenity.

3 Results and discussion

The results of the tooth enamel surface hardness' test before and after applying teeth whitening agents using the Vickers Hardness Tester can be seen in table 1.

Table 1 Mean and standard deviation, T-test, and Wilcoxon of the surface hardness of enamel with white pomegranate extract and 16% carbamide peroxide application

Surface Hardness (VHN)		**Pomegranate Extract**		**Carbamide Peroxide 16%**	
Group	n	Mean ± SD	p-value T-test	Mean ± SD	p-valueWilcoxon
Before Application	5	173.8 ± 28,6	0,331	212.7 ± 76.7	0.043
After Application	5	150.2 ± 32.8		158.2 ± 37.9	

Before performing the Paired Sample t-test analysis, a normality distribution check was conducted by Shapiro Wilk to ensure that the data were normally distributed (p>0.05). For the pomegranate group, Paired Sample t-test with a 5% significance level met p=0.331 (p>0.05). This finding demonstrated no significant difference in the surface hardness of tooth enamel following the application of white pomegranate extract. For the 16 percent carbamide peroxide, normality distribution tests performed before by Shapiro Wilk and indicated that the data were not normally distributed (p<0.05). The Wilcoxon test was used with a 5% significance level, yielding p=0.043 (p<0.05). This finding demonstrated that the enamel's surface hardness decreases following the application of 16 percent carbamide peroxide.

The results of the surface roughness test of tooth enamel before and after the application of teeth whitening agents using the Surface Roughness tester can be seen in table 2.

Table 2 The mean and standard deviation of the surface roughness of the tooth enamel for teeth whitening ingredients, white pomegranate extract, and 16% carbamide peroxide

Surface Roughness (µm)		**Pomegranate Extract**		**16% Carbamide Peroxide**	
Groups	n	Mean ± SD	p-valueT-test	Mean ± SD	p-value Wilcoxon
Before application	5	5.3 ± 4.8	0.029	5.6 ± 4.6	0.043
After application	5	4.3 ± 4.2		4.2 ± 3.9	

Prior to performing the Paired Sample t-test analysis, a normality check was performed prior to and following the application (p>0.05), indicating that the data were normally distributed. We obtained a Paired Sample t-test with a significance level of 5% (p 0.05). This demonstrates that after application of white pomegranate extract, the enamel's surface roughness decreases. Normality tests performed before and after application on the 16 percent carbamide peroxide group (p 0.05) indicated that the data were not normally distributed. Wilcoxon's test with a 5% significance level obtained p0.05, indicating a decrease in enamel surface roughness following 16 percent carbamide peroxide application.

The results of the test of tooth calcium levels before and after the application of teeth whitening agents using the SEM-EDS tool can be seen in table 3.

Before Paired Sample t-test analysis, a normality test was performed before and after the application (p > 0.05), indicating that the data was normally distributed. Paired Sample t-test with a significance level of 5% was obtained (p>

0.05), this indicates that there was no decrease in calcium levels after the application of white pomegranate extract or 16% carbamide peroxide (p>0.05).

Table 3 Mean values and standard deviation of calcium levels in teeth whitening ingredients white pomegranate extract and 16% Carbamide Peroxide

Calcium content (%)		**Pomegranate Extract**		**16% Carbamide Peroxide**	
Groups	n	Mean ± SD	p-value *T-test*	Mean ± SD	p-value *T-test*
Before application	5	68.9 ± 0.5	0.128	69 ± 0.16	0.342
After application	5	75.9 ± 8.5		70.1 ± 2.2	

There was no significant difference in the surface hardness of tooth enamel before and after white pomegranate extract application. This is possible because white pomegranate extract contains sufficient calcium and phosphorus minerals to remineralize tooth enamel. White pomegranate contains flavonoids, which help to reduce demineralization by inhibiting mineral release from the teeth (7,8). There was a decrease in enamel surface hardness in the control group. This is in line with previous research conducted by De Carvalho et al. (2020), namely, the use of carbamide peroxide in bovine can cause a decrease in the surface hardness of tooth enamel. Demineralization of tooth enamel results in a decrease in the surface hardness of tooth enamel. Enamel demineralization is the breakdown of the primary component of tooth enamel, hydroxyapatite. Demineralization occurs when the pH near the enamel surface falls below 5.5, and the acid concentration outside the enamel surface is greater than the acid concentration inside the enamel surface (9). Demineralization occurs due to the difference in the concentration of the teeth whitening agent's acidity on the tooth enamel's surface and inside the tooth enamel. When the enamel is in contact with the bleaching agent, hydrogen ions (H+) will diffuse into the surface of the tooth enamel so that demineralization will occur, which causes the hydroxyapatite to dissolve in the tooth enamel, which will release ion Ca2+, PO49-, OH-. These ions will react with the hydrogen ions (H+) and anion ions in the acid, forming complex compounds that dissolve and cause mineral loss from the tooth enamel surface (10). Continuous demineralization of teeth results in porosity on the surface of the enamel, lowering the surface hardness of the enamel and making it brittle, making it susceptible to wear and caries (11).

There was a decrease in enamel surface roughness in the treatment and control groups, indicating a significant difference between the enamel surface roughness before and after application using white pomegranate extract and 16% carbamide peroxide (p<0.05). The decrease in roughness in the control group, given the application of 16% carbamide peroxide, is in line with the study conducted by De Carvalho et al. (2020) showed a decrease in enamel roughness after the application of 20% carbamide. The decrease in enamel surface roughness in the treatment and control groups was due to remineralization.

The use of white pomegranate extract did not reduce the calcium level of the teeth. The control group showed no significant difference in calcium levels before and after 16% carbamide peroxide application(p>0.05). There was no decrease in calcium levels in the treatment group and control group due to tooth remineralization. Remineralization is the process of returning loose tooth minerals and returning to form hydroxyapatite enamel crystals (12). Wang concluded in 2011 that saliva could be used to restore the mineral component of apatite in acid-exposed enamel. Saliva is critical in maintaining or creating an active environment that promotes demineralization and remineralization of bleached tooth surfaces (13). Saliva will immediately neutralize the resulting acidic environment; saliva also includes calcium and phosphate ions, which will help remineralize the enamel surface (14). Numerous studies have concluded that the application of teeth whitening chemicals and their subsequent storage in saliva results in the remineralization of enamel (15). Numerous processes might enhance saliva's protective role against erosion. For example, saliva works directly on erosion agents by liquefying, cleansing, neutralizing, and buffering them. Second, the organic components of saliva can produce a thin pellicle on the enamel surface, preventing the acid from coming into direct contact with the tooth surface and influencing the degree of tooth disintegration. Thirdly, saliva works as a natural source of new crystal formation when calcium and phosphate ions are present (14). Since saliva performs these three activities, saliva can result in the restoration of tooth enamel from the remineralization process.

From this study it was found that the use of white p as an alternative ingredient for teeth whitening, because it does not reduce surface hardness, produces a smoother surface (reduces surface roughness) and does not decrease calcium levels in tooth enamel.

4 Conclusion

White pomegranate extract can be used as an alternative external bleaching agent because it does not reduce surface hardness. Despite reducing the surface roughness, it does not reduce calcium levels.

Compliance with ethical standards

Acknowledgments

The study did not receive any funding. We wish to thank all study participants for their valuable co-operation.

Disclosure of conflict of interest

The authors of this manuscript do not have any financial or personal conflicts of interest.

Statement of ethical approval

The study received ethical approval by Universitas Airlangga Faculty of dental Medicine Health Research Ethical Clearance Commission, Surabaya (535/HRECC.FODM/IX/2021)

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