RESEARCH PAPER

Comparative Evaluation Of The Effect Of Three Antioxidants On The Calcium-Phosphorous Ratio Of Enamel Bleached With 35% Hydrogen Peroxide Using Energy Dispersive X-Ray Analysis: An In-Vitro Study

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ABSTRACT

Aim: To compare and evaluate the changes in weight percentage of calcium, phosphorus and their ratios in enamel surface bleached with 35% hydrogen peroxide and subsequent reversal with antioxidants-sodium ascorbate, white tea and oregano, using energy dispersive x-ray analysis.

Materials And Methods: Thirty non-carious, freshly extracted human permanent maxillary incisors devoid of visible defects were selected as samples for this study and categorized into three groups (n=10) as follows: Group I – 10% Sodium Ascorbate solution, Group II – 10% White tea solution, and Group III – 5% oregano solution.

Labial surface of each tooth was divided into three sections, of which one portion served as baseline (Sound Enamel), the second portion as bleached enamel and the third portion served as Antioxidant-treated bleached (AOTB) enamel. 35% hydrogen peroxide activated by fast halogen curing light was the protocol followed for bleaching. After antioxidant treatment of portion, the weight percentage of calcium and phosphorus in sound, bleached, and antioxidant-treated bleached enamel was assessed by Energy Dispersive X-ray Analysis.

Results: Data were analyzed with the Shapiro-Wilk test ($p>0.05$) followed by One-way ANOVA and Tukeys HSD Post hoc test. All the samples subjected to bleaching using 35% hydrogen peroxide shows increase in mean calcium and phosphorus weight percentage resulting in a statistically significant decrease in the Ca-P ratio as compared to the sound enamel (P-value<0.01).

Conclusion: Treatment with all three antioxidants prevented the depletion of calcium and phosphorus from the enamel, thereby preventing its ratio alteration.

Keywords: Tooth Bleaching, Antioxidants, EDAX, Sodium Ascorbate, White Tea, Oregano

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1. INTRODUCTION

An extrinsic stain mostly arises from the deposition of chromogenic substances on the tooth's surface. The factors contributing to extrinsic staining include inadequate dental hygiene, consumption of chromogenic foods and beverages, and tobacco usage. The stains are primarily localised to the pellicle and result either from the interaction between sugars and amino acids or from the accumulation of external chromophores within the pellicle. 1 Bleaching is one of the conservative methods for removing mild to moderate extrinsic stains and can also be used to reduce the severity of stains before veneering.2

The agents utilised for dental bleaching are hydrogen peroxide and carbamide peroxide. 35% hydrogen peroxide is used for removing extrinsic stains effectively, as it is capable of penetrating tooth structures by diffusing through the enamel and dentin. Free radicals are released, which react with pigmented molecules and break their double bond

and oxidise chromophore molecules by means of redox reactions.[3]

The most common side effects of bleaching are dentin hypersensitivity and gingival irritation. [4]. Bleaching alters the morphology of enamel and dentin at the microscopic level and also the mineral content. The detrimental impacts on the enamel surface, including heightened porosity of the superficial enamel structure, demineralisation, reduced protein concentration, degradation of the organic matrix, alteration in the calcium-phosphorus ratio, increased depth of enamel grooves, shallow depressions, and minor erosion, were observed when the pH of H_2O_2 decreased from 5.2 to 4.5.[21] Moreover, the enduring peroxide effect enhances the solubility of inorganic ions and modifies their original proportions, thereby reducing the Ca-P ratio of enamel. Therefore, it is essential to prevent the modification of the Ca-P ratio by eliminating the enduring peroxide impact.

Prior literature has indicated that the persistent effects of peroxides can be mitigated by the prompt administration of antioxidants. 22, 23 Antioxidants eliminate peroxides and residual free radicals that compromise the bonding procedures.

The molecular components of dental hard tissues were evaluated using several techniques, including infrared spectroscopy, electronic microprobe analysis, Raman spectroscopy, and energy-dispersive X-ray analysis (EDAX). X-ray microanalysis employing EDAX is commonly utilised in the examination of matrix elements such as calcium and phosphorus.

This study was designed to investigate the effects of three antioxidants – 10% sodium ascorbate solution, 10% white tea solution, and 5% oregano solution – on the calcium-phosphorus ratio of enamel surfaces bleached with 35% hydrogen peroxide, utilising energy-dispersive X-ray analysis.

2. MATERIALS AND METHODS

PREPARATION OF THE ANTIOXIDANTS

PREPARATION OF 10% SODIUM ASCORBATE SOLUTION

10 grams of sodium ascorbate powder (S D Fine-Chem. Limited, Mumbai, India) was mixed in 100 millilitres of distilled water to prepare a 10% sodium ascorbate solution.

PREPARATION OF 10% WHITE TEA SOLUTION

10g of white tea powder is dissolved in 100ml of boiling water and filtered to get 10% aqueous extract of white tea.

PREPARATION OF 5% OREGANO SOLUTION

Ground leaves were macerated in 100 ml of ethanol at ambient temperature for 24 hours. The solution was concentrated using a rotary evaporator, yielding a 5% oregano oil solution.24

Thirty maxillary incisors were obtained from the Department of Oral and Maxillofacial Surgery. The samples were excised from residual tissue tags, rinsed in running tap water, and preserved in a 0.1% thymol solution at 4ºC until use. The criteria for sample selection included teeth extracted for periodontal reasons, devoid of caries or restorations, unexposed to any chemical agents prior to extraction, and free from cracks or fractures. Samples exhibiting stains, morphological or structural anomalies, discolourations, and other discernible faults were excluded. The samples were rinsed with running water to remove thymol residue and were stored in artificial saliva, except for bleaching and testing operations. The 30 samples were categorised into 3 groups (n=10) according to the antioxidant treatment administered as follows:

Group I: Administration of a 10% sodium ascorbate solution

Group II: Administration of a 10% white tea solution

Group III: Administration of 5% oregano solution

Labial enamel surfaces of all the samples were divided into three portions. One portion of the sample was coated with acid-resistant nail varnish leaving the other two portions unprotected. Bleaching was performed on the unprotected portions using 35% hydrogen peroxide (Polo office), and subjected to rapid halogen-curing light (Whitening accelerator, C-Bright-I at 3000 mW/cm²) for 15 minutes. The gel was removed from the tooth surface, a bleaching chemical was reapplied, activated by light, and left for a further 15 minutes before being rinsed off. After the operation, the second segment of the labial enamel surface was enveloped with acid-resistant nail varnish. The third section that remained exposed on the labial enamel surface underwent the application of antioxidants. 10% Sodium ascorbate gel was applied for the samples in group I, samples in group II received 10% White tea solution and the samples in group III received 5% oregano solution for a time period of 10 minutes. Ultimately, all samples were treated with nail polish remover (acetone) and immersed in 99.9% ethyl alcohol to facilitate drying. The mineral composition of each sample piece was determined using EDAX.

QUANTITATIVE EVALUATION

Analysis Of Ca-P Ratio Using Edax

All samples were subjected to an ion sputter (EMS 7620 Mini Sputter Coater) to eliminate water from the tooth specimens for the assessment of calcium and phosphorus values. The weight % of calcium and phosphorus in baseline (sound), bleached, and antioxidant-treated bleached enamel for all three antioxidant groups was determined using

EDAX. The weight percentages of calcium and phosphorus were subsequently transformed into the Ca-P ratio for each portion of the three antioxidants, and this ratio was derived from the collected data.

STATISTICAL ANALYSIS

The data obtained were tabulated and analyzed using SPSS software version 22. The data were assessed for normal distribution using Shapiro-wilk test (P>0.05). Hence parametric test (one way ANOVA and Tukey HSD post hoc test) was conducted to assess the statistical significance among the three groups.

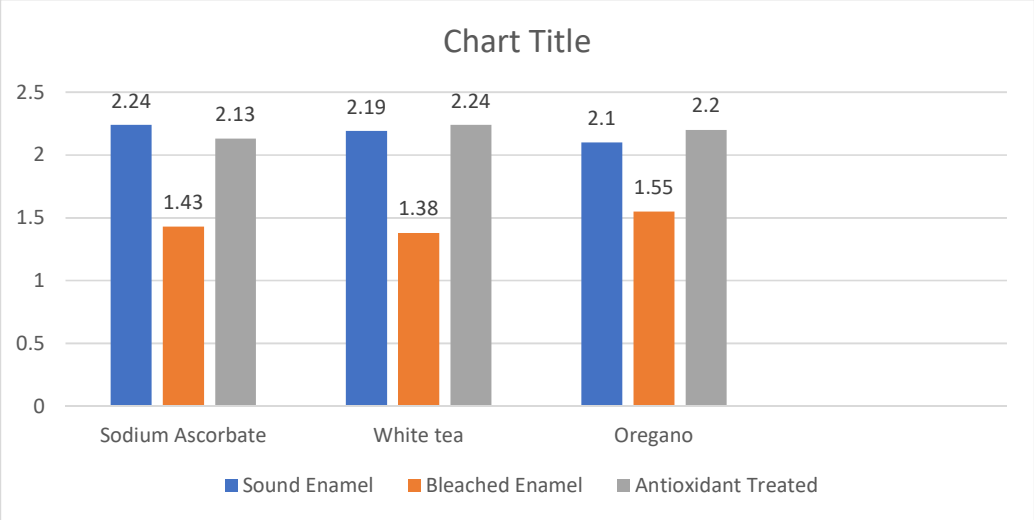
The Ca/P ratio of sound enamel was 2.24, 2.19, and 2.10, respectively for Sodium ascorbate, white tea, and Oregano. The Ca/P ratio in the bleached enamel diminished to 1.43, 1.38, and 1.55 among the three antioxidant-treated groups. This ratio continued to increase, reaching values of 2.13, 2.24, and 2.20 for the three groups. The results indicated that all samples treated with 35% hydrogen peroxide bleaching demonstrated a statistically significant reduction in the Ca/P ratio compared to samples that did not undergo bleaching. The striking finding was that there was a significant increase in the Ca/P ratio on application of all the three antioxidants to the same extent when compared with bleached enamel.

3. RESULT

TABLE I: Mean Calcium, Phosphorous and Ca:P values of Sound, Bleached and Antioxidant Treated Enamel surfaces of all the Three Antioxidant Groups.

Samples	Sodium ascorbate			White tea			Oregano		
	Ca%	P%	Ca/P	Ca%	P%	Ca/P	Ca%	P%	Ca/P
Sound enamel	39.65	17.63	2.24	37.99	17.34	2.19	37.12	17.66	2.10
Bleached enamel	41.28	28.78	1.43	38.25	27.54	1.38	39.53	25.35	1.55
Antioxidant Treated	37.72	17.65	2.13	39.82	17.75	2.24	38.35	17.39	2.20

Graph 1: Graphical representation of mean Calcium, Phosphorous and Ca:P values of Sound, Bleached and Antioxidant Treated Enamel surfaces of all the Three Antioxidant Groups



4. DISCUSSION

Tooth enamel is a highly mineralised tissue, comprising 96% inorganic ingredients, with the remainder consisting of water and organic material. The principal mineral constituent is hydroxyapatite, characterised by the fundamental formula Ca10(PO4)6(OH)2, although additional ions, such as fluoride, are typically integrated.

Bleaching agents emit free radicals in the form of nascent oxygen and hydroxyl or perihydroxyl ions upon application to dental structures. These molecules interact with electron-dense areas of pigments inside the tooth matrix, decomposing large pigmented molecules into smaller, less pigmented counterparts. The conversion of carbon double bond chains to hydroxyl groups decreases the quantity of light absorbed. Consequently, the tooth has a lighter shade25.

One of the possible adverse effects of bleaching is weakening of enamel structure by oxidation of organic and inorganic elements. Hydrogen peroxide and the the liberated free radicals may interact with both the organic and inorganic components of the enamel leading to morphological changes26. Rotstein et al. revealed that bleaching agents alter the amounts of calcium and phosphorus in hydroxyapatite crystals, the fundamental components of dental hard tissues, and also documented a reduction in strength and increased solubility of enamel, dentin, and cementum following bleaching.27

Due to the persistent oxidative effect of hydrogen peroxide on the enamel surfaces after bleaching, there is an increase in the solubility of ions that lead to change in the original ratio of inorganic components of the enamel. 28 The remnant is detrimental to the adhesion of resinous materials because its residual oxygen disrupts resin bonding and inhibits polymerisation.29 Therefore, a waiting period is advised before applying composite materials to the bleached enamel surface.21

To overcome this, numerous antioxidants, including guava seed extract, green tea extract, sodium ascorbate, pomegranate peel extract, α-tocopherol, oregano, and grape seed extract, have been utilised for their oxygen scavenging properties to neutralise free radicals.30,31 Several studies have determined that the application of antioxidants post-bleaching enhances immediate bonding and reinstates the bond strength of composite resin material to enamel.32,22

The mean calcium and phosphorous ratio of the enamel surface were detected using energy dispersive X-ray analysis. Bleaching was performed twice with a 15-minute gap. After 24 hours, the samples were assessed using EDAX, and the mean calcium and phosphorus ratio was found to decrease from baseline in all three groups. This is because of the dissolution of Ca and P ions by the persistent peroxide effect and it is evident increase in mean calcium and phosphorus wt% and this reduction is significant when compared to baseline enamel. The drastic decrease in Ca-P ratio after bleaching is due to a greater amount of phosphorus ions dissolution compared to calcium ions. The cause may be attributed to the weaker bonding of the phosphate moiety, which is covalently attached to the hydroxyapatite crystals, compared to the calcium ions that are ionically bonded.30 These results correlate with the previous study done by Santini et al where bleaching resulted in the depletion of phosphate groups from the surface enamel, accompanied by degradation of the enamel matrix.33

Immediate application of antioxidants after bleaching the mean calcium and phosphorus ratio was maintained to 2.13, 2.24, and 2.20 respectively for all three groups and it is found to be not statistically significant. This outcome was

likely attributable to the oxygen-scavenging properties of the antioxidants, leading to the neutralisation of free radicals.

Sodium ascorbate is a naturally occurring water-soluble antioxidant. It facilitates the maintenance of the calcium and phosphorus ratio without ion dissolution by restoring the modified redox potential of the substrate, thereby reversing the reduced mineral ions.21 Since vitamin C and its salts are non-toxic, they can be applied to dental hard tissues without inducing any adverse biological effects or clinical risks.

Oregano is a therapeutic herb native to dry rocks. Oregano extract is a potent natural antioxidant and antibacterial34 due to the presence of carvacrol and thymol, Monoterpene hydrocarbons and phenolic compounds. The suggested antioxidant mechanism of thymol is evidenced by a concentration-dependent reduction in cell count, deterioration of cell shape, and a decline in total protein content in an in vitro research.35 Thymol may induce apoptosis in human cells through mitochondrial pathways.36

Saponins, polysaccharides, and polyphenols are particularly abundant in white tea. These bioactive ingredients offer numerous health advantages, such as anti-aging, anti-inflammatory, and antioxidant qualities. White tea's polyphenolic constituents have strong antioxidative qualities and efficiently scavenge reactive oxygen species.37

This study reveals no significant difference in the efficacy of three antioxidants in avoiding changes to the Ca-P ratio. All three antioxidants shown comparable efficacy in inhibiting the continued dissolution of ions and modification of the Ca-P ratio in bleached enamel.

5. CONCLUSION

Within the limitations of this current research, it can be concluded that

The dissolution of calcium and phosphate ions results from the enduring peroxide impact following bleaching.

Bleaching with 35% hydrogen peroxide enhances the solubility of calcium and phosphorus ions while reducing the Ca-P ratio.

The prompt administration of antioxidants post-bleaching effectively reinstates the modified potential and preserves their ratios.

The effectiveness of the three antioxidants—10% sodium ascorbate solution, white tea extract, and 5% oregano extract—was comparable

REFERENCES

1. Viscio D, Gaffar A, Fakhry-Smith S, Xu T. Present and future technologies of tooth whitening. Compendium of continuing education in dentistry.(Jamesburg, NJ: 1995). Supplement. 2000(28):S36-43.
2. SruthyPrathap, H Rajesh Vinitha et al. Extrinsic stains and management: A new insight. J. Acad Indus. Res 2013: 1(8).
3. Borges AB, Torres CR, De Souza PA, Caneppele TM, Santos LF, Magalhaes AC. Bleaching gels containing calcium and fluoride: effect on enamel erosion susceptibility. International journal of dentistry. 2012;2012.
4. Leonard Jr RH, Bentley C, Eagle JC, Garland GE. Nightguard vital bleaching: a long-term study on efficacy, shade retention, side effects, and patients' perceptions. Journal of Esthetic and Restorative Dentistry. 2001;13(6):357.
5. Abouassi T, Wolkewitz M, Hahn P. Effect of carbamide peroxide and hydrogen peroxide on enamel surface: an in vitro study. Clinical oral investigations. 2011 Oct 1;15(5):673-80.
6. Azrak B, Callaway A, Kurth P, Willershausen B. Influence of bleaching agents on surface roughness of sound or eroded dental enamel specimens. Journal of Esthetic and Restorative Dentistry. 2010 Dec 1;22(6):391-9.
7. Ben-Amar A, Liberman R, Gorfil C, Bernstein Y. Effect of mouthguard bleaching on enamel surface. American Journal of Dentistry. 1995 Feb;8(1):29-32.
8. Bitter NC. A scanning electron microscopy study of the effect of bleaching agents on enamel: a preliminary report. The Journal of prosthetic dentistry. 1992 Jun 1;67(6):852-5.
9. Bitter NC. A scanning electron microscope study of the long-term effect of bleaching agents on the enamel surface in vivo. General Dentistry. 1998;46(1):84-8.
10. Cadenaro M, Navarra CO, Mazzoni A, Nucci C, Matis BA, Di Lenarda R, Breschi L. An in vivo study of the effect of a 38 percent hydrogen peroxide in-office whitening agent on enamel. The Journal of the American Dental Association. 2010 Apr 1;141(4):449-54.
11. Ernst CP, Marroquin BB, Willershausen-Zönnchen B. Effects of hydrogen peroxide-containing bleaching agents on the morphology of human enamel. Quintessence International. 1996 Jan 1;27(1).
12. Güorgan S, Bolay S, Alacam R. In vitro adherence of bacteria to bleached or unbleached enamel surfaces. Journal of oral rehabilitation. 1997 Aug;24(8):624-7.
13. Haywood VB, Leech T, Heymann HO, Crumpler D, Bruggers K. Nightguard vital bleaching: effects on enamel surface texture and diffusion. Quintessence International. 1990 Oct 1;21(10).
14. Hegedüs C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. Journal of dentistry. 1999 Sep 1;27(7):509-15.
15. Hunsaker KJ, Christensen GJ, Christensen RP.

Tooth bleaching chemicals-influence on teeth and restorations. InJournal of Dental Research 1990 Mar 1 (Vol. 69, pp. 303-303). 1619 DUKE ST, ALEXANDRIA, VA 22314: AMER ASSOC DENTAL RESEARCH.
16. Sa Y, Sun L, Wang Z, Ma X, Liang S, Xing W, Jiang T, Wang Y. Effects of two in-office bleaching agents with different pH on the structure of human enamel: an in situ and in vitro study. Operative dentistry. 2013 Jan;38(1):100-10.
17. Smidt A, Feuerstein O, Topel M. Mechanical, morphologic, and chemical effects of carbamide peroxide bleaching agents on human enamel in situ. Quintessence International. 2011 May 1;42(5).
18. Sun L, Liang S, Sa Y, Wang Z, Ma X, Jiang T, Wang Y. Surface alteration of human tooth enamel subjected to acidic and neutral 30% hydrogen peroxide. Journal of dentistry. 2011 Oct 1;39(10):686-92.
19. Titley KC, Torneck CD, Ruse ND. The effect of carbamide-peroxide gel on the shear bond strength of a microfil resin to bovine enamel. Journal of Dental Research. 1992 Jan;71(1):20-4.
20. Xu B, Li Q, Wang Y. Effects of pH values of hydrogen peroxide bleaching agents on enamel surface properties. Operative dentistry. 2011 Oct;36(5):554-62.
21. Josey AL, Meyers IA, Romaniuk K, Symons AL. The effect of a vital bleaching technique on enamel surface morphology and the bonding of composite resin to enamel. Journal of oral rehabilitation. 1996 Apr;23(4):244-50.
22. Lai SC, Mak YF, Cheung GS, Osorio R, Toledano M, Carvalho RM, Tay FR, Pashley DH. Reversal of compromised bonding to oxidized etched dentin. Journal of Dental Research. 2001 Oct;80(10):1919-24.
23. Vidhya S, Srinivasulu S, Sujatha M, Mahalaxmi S. Effect of grape seed extract on the bond strength of bleached enamel. Operative dentistry. 2011 Jul;36(4):433-8.
24. John Coccimiglio, MisaghAlipour, Zi-Hua Jiang et al. Antioxidant, Antibacterial and cytotoxic activities of the ethanolic Origanum Vulgare extract and its major constituents. 2016
25. Fearon J. Tooth whitening: concepts and controversies. J IrDent Assoc. 2007 Sep 1;53(3) :132-40.
26. Poorni S, Kumar RA, Shankar P, Indira R, Ramachandran S.Effect of 10% sodium ascorbate on the calcium: Phosphorusratio of enamel bleached with 35% hydrogen peroxide: an invitro quantitative energy-dispersive X- ray analysis. Contemporary clinical dentistry. 2010 Oct ;1(4):223.
27. Rotstein I, Lehr Z, Gedalia I. Effect of bleaching agents on inorganic components of human dentin and cementum. Journal of Endodontics. 1992 Jun 1;18(6):290-3.
28. Madison S, Wal ton R. Cervical root resorption following bleaching of endodontically treated teeth. Journal of Endodontics.1990 Dec 1;16(12):570 -4.
29. Kaya AD, Türkün M, Arici M. Reversal of compromised bonding in bleached enamel using antioxidant gel. Operative dentistry. 2008 Jul ;33(4):441-7.
30. Gogia H, Taneja S, Kumar M, Soi S. Effect of different antioxidants on reversing compromised resin bond strength after enamel bleaching: An in vitro study. Journal of
31. Conservative dentistry: JCD. 2018 Jan;21(1):100.
32. Sharafeddin F, Motamedi M, Modiri S. Effect of Immediate Application of Pomegranate Peel , Grape Seed and Green Tea Extracts on Composite Shear Bond Strength of In -Office Bleached Enamel. Research Journal of Biological Sciences.
33. 2013;8(3) :83-7.
34. Ozel in AA, Guiraldo RD, Carvalho RV, Lopes MB, Berger SB. Effects of green tea application time on bond strength after enamel bleaching. Brazilian dental journal. 2014Oct ;25(5):399-403.
35. Santini A, Pulham CR, Rajab A, Ibbetson R. The effect of a10% carbamide peroxide bleaching agent on the phosphate concentration of tooth enamel assessed by Raman spectroscopy. Dental Traumatology. 2008 Apr;24(2):220 -3.
36. John Coccimiglio, Misagh Alipour, Zi-Hua Jiang, Christine Gottardo, and Zacharias Suntres, Antioxidant, Antibacterial, and Cytotoxic Activities of the Ethanolic Origanum vulgare Extract and Its Major Constituents. Oxidative Medicine and Cellular Longevity, Volume 2016, Article ID 1404505, 8 pages
37. A. T. Koparal and M. Zeytinoglu, “Effects of carvacrol on ahuman non-small cell lung cancer (NSCLC) cell line, A549,”Cytotechnology, vol. 43, no. 1–3, pp. 149–154, 2003
38. H.-T. Chang, S.-S. Hsu, C.-T. Chou et al., “Effect of thymol onCa2+ homeostasis and viability in MG63 human osteosarcomacells,” Pharmacology, vol. 88, no. 3-4, pp. 201–212, 2011.
39. Wijesekara T, Liu Y, Xu B. New insights into chemical compositions and health benefits of white tea and development of new products derived from white tea. Food Science and Human Wellness, 2025, 14(11): 9250424.