EVALUATION OF THE EFFECT OF ANTICAY APPLICATION ON ENAMEL MICROHARDNESS OF TEETH BLEACHED WITH TWO DIFFERENT AGENTS: AN IN-VITRO STUDY

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Abstract

Context- The desire for whiter, younger-looking, healthier teeth has raised the market for dental whitening. Carbamide peroxide is commonly used in home bleaching procedures as it is safer and causes fewer side effects. Strawberries are one of the natural components that are now utilised to whiten discoloured teeth as it contains both ellagic and malic acids. However bleaching agents results in mineral loss resulting in decreased enamel microhardness. After bleaching, remineralizing therapy using a remineralizing chemical like anticay can restore the enamel's lost surface hardness.
Aim- The aim of this invitro study was to evaluate the effect of anticay on the microhardness of enamel after bleaching with carbamide peroxide and strawberry extract.
Materials and methods- Twenty-five freshly extracted teeth decoronated and divided mesiodistally into two halves to obtain a total of fifty samples. Samples were randomly divided into three groups as follows.
Group 1: Control group(n=10)
Group 2: (n=20)
Subgroup 2A (n=10): Bleaching with 10% carbamide peroxide
Subgroup 2B (n=10): Bleaching with 10% Carbamide peroxide and an additional Anticay application for 1 minute after treatment.
Group 3: (n=20)
Subgroup 3A (n=10): Bleaching with 2% strawberry gel
Subgroup 3B (n=10): Bleaching with 2% strawberry gel and an additional Anticay application for 1 minute after treatment
The samples were subjected to indentation to test the microhardness using Knoops hardness analyser.
Statistical Analysis- Data was analysed using Student t test and ANOVA. p value <0.05.
Results- It was observed that Group 1 has maximum microhardness value (369.80) whereas Subgroup 2A shows lowest microhardness value (285.20). A highly significant difference in the values of microhardness was seen between Subgroups treated with and without anticay application. Subgroups subjected to anticay application post bleaching showed higher microhardness value than Subgroups without anticay application. [**p - value < 0.001**]. Thus, it indicates that application of anticay post bleaching with carbamide peroxide and strawberry gel significantly increased the microhardness when compared to carbamide peroxide and strawberry gel used alone.
Conclusion- The application of remineralizing agent containing calcium sucrose phosphate/anticay after bleaching increased the microhardness of enamel.

Keywords- Carbamide peroxide, Strawberry gel, Anticay, bleaching, enamel microhardness

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1. Introduction

In addition to treating dental disorders, modern dentistry deals a growing number of patient requests for aesthetic improvements.[1] Indeed, aesthetic features will be impacted by any discoloration that may develop in enamel or dentin.[2] Today's dentist offers a wide range of procedures to improve the appearance of a smile with stained teeth.[3] To treat these tooth discolorations, the range of therapies goes from crowns, veneers and placement of direct restorations to minimally invasive treatments including bleaching, macroabrasion and microabrasion to just prophylaxis. However, bleaching has been regarded as a safe, efficient, minimally invasive, and non-destructive procedure answering both the clinicians and patients question to a safe conservative approach.[3]

Bleaching is a process of breakdown of complex, high molecular weight organic molecules into simpler, low molecular weight molecules with lower colour reflectance is what causes the colour shift in enamel and dentin after hydrogen peroxide passes through the enamel.[4] Its effects are not limited, though, as it may also lead to protein deterioration and mineral loss.[5]

Carbamide peroxide (CP) is the most commonly used bleaching agent in at home bleaching techniques as it has fewer adverse effects. Carbamide peroxide is a precursor and acts by breaking down into urea and hydrogen peroxide. However, its use is still debatable due to its effect on oral cavity.

Recently there has been an upsurge in usage of natural substances for treatment of various diseases as they minimise or prevent the side effects.[6] Similarly, this has led to a constant search for replacing the existing chemicals in cosmetic dentistry used for various purposes. This change would be welcomed in dentistry if there is an alternative for CP due to its various adverse effects on the enamel surface.

The natural bleaching agent strawberry is being utilised to whiten discoloured teeth as it contains both ellagic acid and malic acid, both of which can whiten teeth.[7,8] These acids have the ability to bind calcium in teeth, resulting in enamel crystal porosity and dental erosion, which further reduces the hardness of the enamel.[7]

The morphology and structure of teeth may be compromised by the peroxide molecules' mechanism of action.[9] The mineral content of enamel and dentin may be decreased by the acidic pH levels of bleaching agents. These effects intensify with the duration of the treatment and are accompanied by progressive changes in calcium and phosphorus composition.[10] It causes changes in the enamel microhardness and surface roughness, and these alterations might encourage the formation of plaque.[11]

In order to maintain dental health and the capacity to withstand masticatory, mechanical, and chemical stresses, enamel microhardness must be preserved. This raises some concerns about bleaching and the integrity of the tooth structure.[12] The introduction of agents that can make up for the mineral loss could help restore the mechanical qualities of enamel that were lost after the application of bleaching treatments. According to studies, using substances like fluoride, casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and calcium sucrose phosphate (Anticay) helps the tooth structure remineralize.

Anticay, a Calcium Sucrose Phosphate (CaSP)–calcium orthophosphate complex, supplies both calcium and phosphate in a soluble form. It is a fine, white, non-hygroscopic powder with a neutral blend taste. It contains approximately 11.5% calcium on a dry weight basis. This complex helps reduce the acid solubility of enamel and increase the rate of remineralization by a common ion effect. Furthermore, it has shown to inhibit the formation and adherence of plaque to the enamel surface.[13] It is completely soluble in water at all pH values and hence able to provide high concentrates of calcium and phosphate ions in the oral cavity. It has been proposed that one percent anticay solution contains about 30×10^{-3} M calcium (Ca) and 10×10^{-4} M SP ions. Saliva contains about 1.4×10^{-3} M calcium and 4×10^{-3} M phosphate (P) ions. High concentration of calcium and phosphate in the presence of anticay facilitates rapid remineralization of enamel.[14]

The purpose of this study was to evaluate the effect of anticay on the microhardness of enamel after bleaching with carbamide peroxide and strawberry extract. The null hypothesis states that the microhardness of enamel does not increase after the use of anticay.

2. Materials and Method

Twenty-five human permanent maxillary premolars extracted for periodontal reasons and orthodontic reasons were collected and visually inspected to exclude those with cracks or defects or teeth with hypoplastic lesions or fluorosis. All soft tissue remnants on root surface were cleaned and debris removed with the help of ultrasonic scaler and teeth were subjected to surface disinfection by completely immersing in 2.5% Sodium hypochlorite, after which teeth were stored in distilled water until use.

Each sample was decoronated at cementoenamel junction using a water-cooled diamond disc and then crowns were vertically sectioned in two equal halves mesio-distally to obtain a total of fifty samples. The buccal and palatal/lingual halves of each crown was mounted in an acrylic resin of standard dimension of

10 mm × 10 mm × 5 mm such that the enamel surface faced upward. The samples were kept in cold water until complete curing of the resin to avoid thermal effects generated by the resin during curing process. The enamel surface of teeth was grounded to obtain a flat surface using water cooled abrasive wheel in a sequence of 120, 240 and 400 grit silicon carbide paper.

The samples were randomly divided into the following three treatment groups:

GROUPS	**BLEACHING TREATMENT** (Application time: 3 hours for 2 weeks)	**ANTICAY APPLICATION** (ToothMin) (Application time: 1 minute)
Group 1 (n=10)	Control group	No
Group 2 (n=20)		
Subgroup 2A (n=10)	16% Carbamide Peroxide (Pola Night SDI)	No
Subgroup 2B (n=10)	16% Carbamide Peroxide (Pola Night SDI)	Yes
Group 3 (n=20)		
Subgroup 3A (n=10)	Strawberry gel	No
Subgroup 3B (n=10)	Strawberry gel	Yes

Strawberry gel preparation

The fresh pulpy strawberry (Fragaria ananassa) was procured from the market. A 200 gm of strawberry fruits were cleaned, cut into cubes and blended with 15 mL of distilled water in a blender to obtain about 100 mL of strawberry concentrate. This concentrate was filtered and then transferred into a cooling centrifuge to be processed at 2000 rpm for about 20 minutes at a temperature of 40º C. The clear liquid thus collected (2% SE) was stored at 4º C till further use.

Treatment protocol

Bleaching agent was applied onto the exposed enamel surface of the samples for 3 hours a day for 14 consecutive days in the respective groups. Then the samples were rinsed with air water spray for 30 seconds and air dried after each treatment. In Subgroup 2B and Subgroup 3B, samples were subjected to additional application of ToothMin paste for 1 min after each bleaching treatment. Then the samples were rinsed off and dried using air water spray and all the samples were immersed in distilled water for the remaining hours.

Determination of microhardness

The surface hardness of each sample was determined with a Knoops hardness tester with 100 g load, which was used to make indentations on the enamel surface. The loaded diamond was allowed to sink and rest on the enamel surface for 15 s and the Knoops hardness number was determined and recorded.

Statistical Analysis

Descriptive and inferential statistical analyses were carried out in the present study. Results on continuous measurements were presented on Mean ± SD. Level of significance was fixed at p=0.05 and any value less than or equal to 0.05 was considered to be statistically significant.

Student t tests (two tailed, unpaired) was used to find the significance of study parameters on continuous scale between two groups. Analysis of variance (ANOVA) was used to find the significance of study parameters between the groups (Inter group analysis). Further post hoc analysis was carried out if the values of ANOVA test were significant.

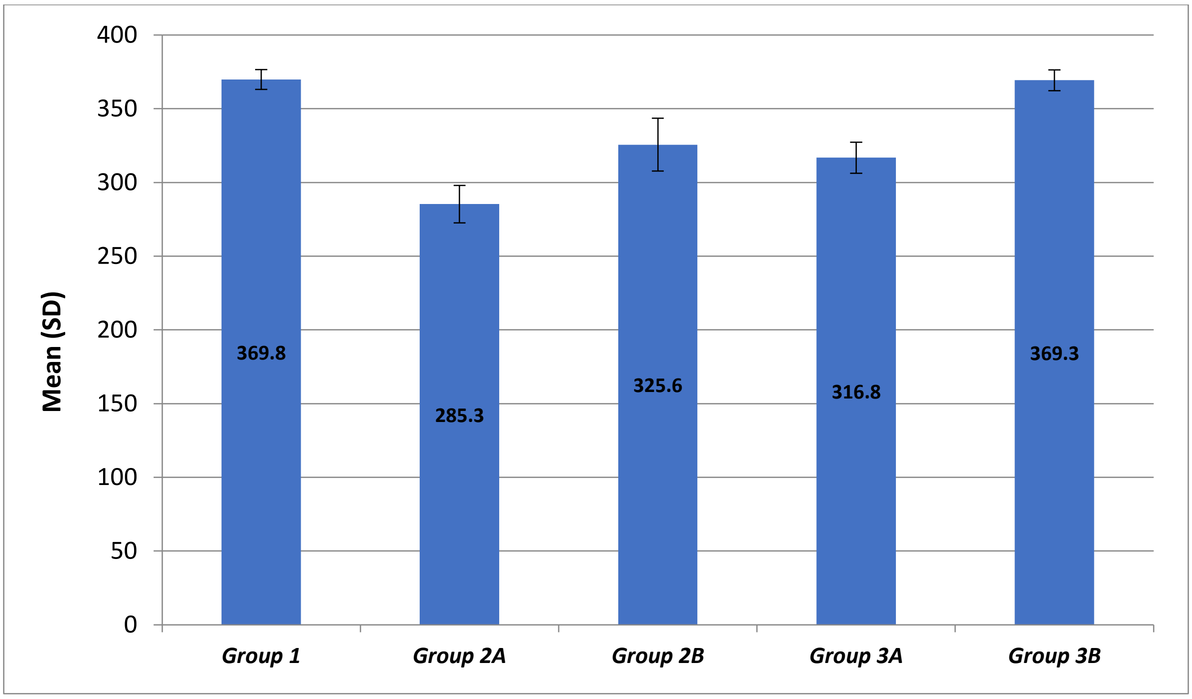
The Statistical software IBM SPSS statistics 20.0 (IBM Corporation, Armonk, NY, USA) was used for the analyses of the data and Microsoft word and Excel were used to generate graphs, tables.

3. Results

Comparison of enamel microhardness in terms of their mean values and standard deviation was done using ANOVA. It was observed that Group 1 has maximum microhardness value (369.80) whereas Subgroup 2A shows lowest microhardness value (285.20). (Table 1 and Graph 1)

Group	N	Mean	Std. Deviation	F value	P value
Group 1	10	369.80	6.795	**95.577**	**<0.001****
Subgroup 2A	10	285.30	12.650		
Subgroup 2B	10	325.60	17.939		
Subgroup 3A	10	316.80	10.581		
Subgroup 3B	10	369.30	6.961		
Total	50	333.36	34.651		

Table 1: Comparison of the enamel microhardness in terms of {Mean (SD)} among all the groups using ANOVA test



Graph 1: Comparison of the enamel microhardness of all the groups in terms of {Mean (SD)} using ANOVA test

Table 2 shows highly significant difference in the values of microhardness between Subgroup 2A and Subgroup 2B. Subgroup 2B (Carbamide Peroxide and Anticay, 325.60) showed higher microhardness value than Subgroup 2A (Carbamide Peroxide, 285.30). [**p - value < 0.001**]. Thus it indicates that application of anticay post bleaching with carbamide peroxide significantly increased the microhardness when compared to carbamide peroxide used alone.

Group 2	N	Mean	Std. Deviation	t value	P value
Subgroup 2A	10	285.30	12.650	**5.806**	**<0.001****
SubGroup 2B	10	325.60	17.939		

Table 2: Comparison of the enamel microhardness of group 2 in terms of {Mean (SD)} between both the subgroups using unpaired t test

Table 3 shows mean values and standard deviation of microhardness for Subgroup 3A and Subgroup 3B. The intragroup comparison revealed highly significant difference in the values of microhardness in both the Subgroups. Subgroup 3B (Strawberry and Anticay,369.30) showed higher microhardness value than Subgroup 3A (Strawberry,316.80 mm). [**p - value < 0.001].** Thus, it indicates that application of anticay post bleaching with 2% Strawberry gel significantly increased the microhardness when compared to 2% Strawberry gel used alone.

Group 3	N	Mean	Std. Deviation	t value	P value
Subgroup 3A	10	316.80	10.581	**13.108**	**<0.001****
Subgroup 3B	10	369.30	6.961		

Table 3: Comparison of the enamel microhardness of Group 3 in terms of {Mean (SD)} between both the subgroups using unpaired t test

Table 4 shows mean values and standard deviation of microhardness for Subgroup A2 and Subgroup 3A. The intergroup comparison revealed highly significance difference in the values of microhardness for both the Subgroups. Subgroup 3A (Strawberry,316.80) showed higher microhardness value than Subgroup 2A (Carbamide Peroxide,285.30). [**p - value < 0.001].** Hence it can be concluded that 2% Strawberry gel caused significantly lesser reduction in enamel microhardness when compared to 16% carbamide peroxide.

Group	N	Mean	Std. Deviation	t value	P value
Subgroup 2A	10	285.30	12.650	**6.040**	**<0.001****
Subgroup 3A	10	316.80	10.581		

Table 4: Comparison of the enamel microhardness in terms of {Mean (SD)} between both the groups (Subgroup 2A and Subgroup 3A) using unpaired t test

Table 5 shows mean values and standard deviation of microhardness for Subgroup 2B and Subgroup 3B. The unpaired t test, revealed highly significance difference in the values of microhardness for both the Groups. Subgroup 3B (Strawberry and Anticay, 369.30) showed higher microhardness value than Subgroup 2B (Carbamide Peroxide and Anticay, 325.60). [**p - value < 0.001].**

Group	N	Mean	Std. Deviation	t value	P value
Subgroup 2B	10	325.60	17.939	**7.182**	**<0.001****
Subgroup 3B	10	369.30	6.961		

Table 5: Comparison of the enamel microhardness in terms of {Mean (SD)} between both the groups (Subgroup 2B and Subgroup 3B) using unpaired t test

4. Discussion

Bleaching is a chemical process involving the oxidation of organic material which is broken down to produce less complex molecules. Most of these smaller molecules are lighter in colour than the original larger molecules. In-office and home bleaching gels contain hydrogen peroxide or its precursor, carbamide peroxide, as the active ingredient in concentrations ranging from 3% to 40% of hydrogen peroxide equivalent. Hydrogen peroxide bleaching generally proceeds via the perhydroxyl anion (HO_2^-) or it can give rise to free radical formation, for example, by homolytic cleavage of either an O–H bond or the O–O bond in hydrogen peroxide to give $H^\bullet$ + $^\bullet OOH$ and $2^\bullet OH$ (hydroxyl radical), respectively.[56] Hydrogen peroxide is an oxidizing agent that, as it diffuses into the tooth, dissociates to produce unstable free radicals which are hydroxyl radicals ($HO^\bullet$), perhydroxyl radicals ($HOO^\bullet$), perhydroxyl anions (HOO^-), and superoxide anions ($OO^{\bullet-}$), which will attack organic pigmented molecules in the spaces between the inorganic salts in tooth enamel by attacking double bonds of chromophore molecules within tooth tissues.[15] The change in double-bond conjugation results in smaller, less heavily pigmented constituents, and there will be a shift in the absorption spectrum of chromophore molecules; thus, bleaching of tooth tissues occurs.[16]

Strawberry (fragaria spp.) is a herbaceous perennial fruit plant belonging to the family "Rosaceae" is grown in many countries. Apart from its various benefits exerted on the well-being of an individual, it is been noted for its natural tooth whitening potential due to its low pH and presence of various constituents namely ellagic acid, citric acid and malic acid as stated by Chacko K et al., in (2018) and Aub M (2010). Song and palmer in 2008 have reported that many fruits namely strawberries, lemon, apples, pears etc., contain H_2O_2.[17] Apart from HP, strawberry also has ellagic acid ($C_{14}H_6O_8$) possessing potential OH clusters, these clusters is claimed to act as a powerful oxidiser during tooth whitening process. The OH and H radicals gets released from the ellagic acid and reacts with the organic molecules disrupting the electron conjugation, thereby changing the energy absorption by forming smaller organic molecules with lighter colour.[18] OH clusters in ellagic acid compared to carboxylic group (COOH) clusters (present in other acids) have larger electronegativity and breaks easily to react with the organic molecules of the tooth enamel. The more the ellagic acid in a fruit the more effective is the bleaching process. Ellagic acid in strawberries range to around 0.43-4.64 mg/g dry weight compared to only around 0.13 mg/g in other fruits.[18] These can be substantiated with the results obtained in this study conducted by Rajith Radhakrishnan et al. Hence, strawberry seems to be a good alternative for tooth bleaching.[19]

Malic acid present in strawberry is said to be involved in the erosion process. These acids can bind calcium in tooth enamel and cause porosity crystals that have an effect on the occurrence of dental erosion and decrease enamel surface hardness.[20] It was reported that strawberry fruit juice has a pH of 3. The enamel will begin experiencing erosion when pH value reaches 5.5. This decrease in pH is considered to cause enamel solubility resulting in dental erosion and decrease in dental enamel microhardness.[21,22] Teeth whitening agent can cause changes in the chemical structure as well changes in the superficial texture of tooth enamel resulting in loss of mineral of the dental structure and decreased microhardness. This loss of mechanical properties of enamel after the use of bleaching agents could be regained by incorporation of remineralising agents that can compensate for mineral loss.

Thus, this study was undertaken to evaluate the effect of remineralising agent i.e. anticay on the microhardness of enamel after bleaching with carbamide peroxide and strawberry extract.

Surface microhardness is the most frequently employed technique for evaluating the effects of bleaching products on enamel and dentin.[23] Meredith et al. reported that the Knoop has been the most popular method for measuring microhardness of brittle materials such as enamel and dentin. In the current study, enamel microhardness was evaluated using Knoops microhardness tester where the lowest loads, 100 g for enamel were chosen to prevent errors in optical measurement and the indenter was allowed to dwell over the enamel surface for a time period of 10 seconds which was in accordance with the study conducted by Chanya Chuenarrom et al.[24] The surface of samples were polished to provide smooth surface for proper indentations of the hardness tester and its evaluation under microscope. It also facilitated measurement by removing the outer hypermineralized layers of enamel.[25]

In the current study, enamel microhardness was evaluated and compared post bleaching treatment for the subgroups 2A (16% Carbamide Peroxide) and 3A (Strawberry gel). It revealed reduction in microhardness of both the bleached groups 16% carbamide peroxide (Subgroup 2A) and strawberry gel (Subgroup 3A) when compared to control group. This can be accounted to the acidic properties of the bleaching agents, causing changes in the mineral content of dental hard tissues, leading a decrease in the microhardness values after the bleaching treatment. This was in accordance with the study conducted by Azer et al., 2009, de Arruda et al., 2012 which showed significant decreases in hardness and fracture resistance.

In the present study, 16% carbamide peroxide (Subgroup 2A) showed greater reduction in microhardness than 2%Strawberry gel (Subgroup 3A). This can be explained by differences in the technique used, like bleaching formulation and concentrations. Higher CP concentration in the bleaching gel results in higher and faster decrease of dental enamel microhardness. This alteration has been correlated to the oxidative action of HP on the organic phase of this hard tissue.[26] The reduction in enamel microhardness after bleaching with 16% carbamide peroxide has been seen in previous studies.[27-33]

The strawberry group showed more enamel microhardness when compared to carbamide peroxide group, this can be possibly explained by the acidic pH of the fruit and lower concentration of the strawberry gel. The acidic pH of gel could be the mechanism responsible for reducing the Ca and P contents and surface microhardness of enamel as well. It has been noticed that strawberry fruit juice has a pH of 3 due to its high content of both ellagic and malic acids.[34] Brambert et al. showed that these acids could bind calcium in tooth enamel and cause porosity that decreases enamel surface hardness, consequently increasing enamel surface roughness.[35] The results of current study is in accordance with study conducted by Asmawati et al.[36]

Therefore, as the concentration of both gels used in this study are different, it may be suggested that the greater aggressiveness caused by 16% CP gel in the present study was due to the release of higher HP concentrations compared to the 2% Strawberry gel.

In the current study, application of anticay significantly improved the microhardness of enamel by helping to regain the minerals lost during the bleaching procedure. The study revealed highly significant difference in the values of microhardness for both the Groups. Subgroup 3B (Strawberry and Anticay, 369.30) showed higher microhardness value than Subgroup 2B (Carbamide Peroxide and Anticay, 325.60). This was in accordance with the study conducted by Sargod et al. who reported that twice-daily application of a CaSP toothpaste significantly reduced the depth of enamel lesions produced by subsequent acidic challenges.[37] Enhanced surface smoothness of the enamel was also observed following two weeks of brushing with a CaSP tooth cream.[38] The efficacy of CaSP was further confirmed by George et al.[14] and Gade,[39] who reported significantly increased enamel microhardness in samples treated with CaSP. Kaur et al.[40] further confirmed the promising prospects of CaSP in improving microhardness. According to this study, both CaSP and CPP-ACP increased the microhardness of bleached enamel; however, CaSP tooth cream was a better agent for increasing microhardness.

The quantitative results obtained in this in vitro study, along with previous reports, suggest that CaSP can be an effective treatment for remineralisation of bleached enamel. However, this study has some limitations as it is an in vitro study. Remineralization in the oral cavity is a complex procedure involving a change in pH and replenishment of calcium and phosphate elements. This may not be achieved in the in vitro conditions. Therefore, further studies using these products in in vivo conditions is recommended.

5. Conclusion

Within the limitations of this in-vitro study, it was concluded that:

- Untreated samples showed highest microhardness whereas samples bleached with 16% carbamide peroxide showed least microhardness.
- 16% carbamide peroxide showed greater reduction in enamel microhardness when compared to 2% strawberry gel.

- The application of remineralizing agent containing calcium sucrose phosphate/anticay after bleaching increased the microhardness of enamel.

Further clinical trials supplemented with in vitro studies are needed to authenticate the results for future clinical applications.

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Conflict Of Interest

The authors report no conflict of interest.

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