Effect of vital bleaching with solutions containing different concentrations of hydrogen peroxide and pineapple extract as an additive on human enamel using reflectance spectrophotometer: An in vitro study

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Abstract

Aim: This study aims to evaluate the color change in human enamel bleached with three different concentrations of hydrogen peroxide, containing pineapple extract as an additive in two different timings, using reflectance spectrophotometer.

Background: The study aimed to investigate the bleaching efficacy on natural teeth using natural enzymes.

Materials and Methods: Baseline color values of 10 randomly selected artificially stained incisors were obtained. The specimens were divided into three groups of 20 teeth each: Group 1 – 30% hydrogen peroxide, Group II – 20% hydrogen peroxide, and Group III – 10% hydrogen peroxide. One half of the tooth was bleached with hydrogen peroxide, and other was bleached with hydrogen peroxide and pineapple extract for 20 min (Subgroup A) and 10 min (Subgroup B).

Statistical Analysis: The results were statistically analyzed using student’s t-test.

Results: The mean ∆E values of Group IA (31.62 ± 0.9), Group IIA (29.85 ± 1.2), and Group IIIA (28.65 ± 1.2) showed statistically significant higher values when compared to the mean ∆E values of Group 1A (25.02 ± 1.2), Group IIA (22.86 ± 1.1), and Group IIIA (16.56 ± 1.1). Identical results were obtained in Subgroup B.

Conclusion: The addition of pineapple extract to hydrogen peroxide resulted in effective bleaching.

Keywords: Bromelain enzyme; hydrogen peroxide; pineapple extract; reflectance spectrophotometer; tooth bleaching

INTRODUCTION

Esthetic smile makeovers have become a common entity these days. Delivering a bright smile becomes a part of treatment protocol of such procedures. These procedures would include from bleaching to extensive restorations, where bleaching is a simpler conservative modality of treatment for the management of discolored teeth. Hence, vital bleaching has gained a lot of popularity. Various agents and techniques for vital bleaching have entered the market; the primary ingredient of these agents revolve around the chemistry of hydrogen peroxide. Hydrogen peroxide and its precursor carbamide peroxide have been used in various concentrations ranging from 10% to 38%, either in office or at home to achieve the desired results. Although hydrogen peroxide provides better outstanding results, the related

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clinical side effects remains inevitable. The scientific evidence of change in surface texture, composition, and microhardness of enamel are reported when bleaching is done with hydrogen peroxide.[4-6] Several attempts have been made in achieving better result of bleaching with various agents by reducing the concentration of hydrogen peroxide.

One such attempt is to lower the concentration of hydrogen peroxide by using vegetative enzymes as an additive to achieve the desired results. These enzymes in addition with hydrogen peroxide promote and accelerate the bleaching effect.[7]

This study aims at providing a new formulation containing enzymes obtained from the fruit “pineapple” for teeth bleaching. Pineapple has high content of enzymes such as bromelain, catalase, and polyphenol peroxidase which are hypothesized in providing better result.[8,9] The aim of this in vitro study was to evaluate the role of pineapple natural extract as an additive to various concentration of hydrogen peroxide in bleaching artificially stained human enamel – using reflectance spectrophotometer.

MATERIALS AND METHODS

Specimen collection
For this in vitro study, sixty maxillary central incisors that were extracted due to periodontal disease were collected. The teeth were later examined for visible cracks, caries defects, and decalcifications. The defective teeth were discarded. Later, the teeth were cleaned of calculus and the remaining soft tissue using an ultrasonic scaler (Satelec, India). They were stored in 0.2% thymol, refrigerated at 4°C until use.

Preparation of pineapple extract
Two hundred grams of pineapple (Ananas comosus) were peeled and cut into small pieces. The pieces were smashed and blended in blender with 25 ml of distilled water. The obtained filtrate was further centrifuged at 2000 rpm for 2 min at a temperature of 4°C. The clear liquid was filtered out and refrigerated at 4°C.

Staining of specimen
Artificial staining protocol used for the study was strictly followed as recommended by Sulieman et al.[14] A 2 g tea bag (Tetley, Bengaluru, India) was suspended in 100 ml of boiling deionized water. After 5 min, the solution was cooled to room temperature. The strained solution was used for staining. Staining was achieved by immersing the teeth for 24 h in above-mentioned solution. The roots of all teeth were cut at cementoenamel junction. The crowns were sectioned to equal mesial and distal halves labio-palatally. Using self-cure clear acrylic resin, they were mounted exposing the labial surface.

One half of the tooth was bleached with hydrogen peroxide alone, and the other half of the same tooth was bleached with a combination of hydrogen peroxide and pineapple extract.

Bleaching protocol
The bleaching solution contains 1 ml of pineapple extract + 1 ml of phosphate buffered solution + 28 ml of hydrogen peroxide. They were proportioned using pipette and mixed in a test tube. One half of the tooth was bleached with hydrogen peroxide while the other was bleached in combination with pineapple extract.

The specimens in each subgroup were immersed in experimental solutions for 20 min and 10 min, respectively. After bleaching, the teeth were rinsed and stored in artificial saliva at 37°C for 24 h.

Testing methodology
The testing was done with reflectance spectrophotometer over a white background, which recorded color variables L*, a*, b* in accordance to CIEL*a*b color system using a formula:

\[ \Delta E = (|\Delta L|^2 + |\Delta a|^2 + |\Delta b|^2)^{1/2} \]

Statistical analysis
The obtained data were analyzed using student’s t-test at 5% significance level.

RESULTS
The results of this in vitro study are given in Table 1.
Table 1: Mean ∆E values’ standard deviation

| Time | Group 1 | Group 2 | Group 3 |
|------|---------|---------|---------|
|      | 30% hydrogen peroxide | 30% hydrogen peroxide + extract | 20% hydrogen peroxide | 20% hydrogen peroxide + extract | 10% hydrogen peroxide | 10% hydrogen peroxide + extract |
| A (20 min) | 25.02 ± 1.2 | 31.62 ± 0.9 | 22.86 ± 1.1 | 29.85 ± 1.2 | 16.56 ± 1.1 | 28.65 ± 1.2 |
| B (10 min) | 20.12 ± 0.9 | 25.16 ± 1.3 | 15.14 ± 1.3 | 23.16 ± 0.9 | 10.14 ± 0.8 | 22.56 ± 1.1 |

In all the groups and subgroups evaluated in the study, the mean values obtained with the use of pineapple extract along with hydrogen peroxide showed statistically significant whitening when compared to the specimens that were bleached only with hydrogen peroxide (P < 0.05).

Comparing within the groups, the mean values obtained with the use of 30%, 20%, and 10% hydrogen peroxide along with pineapple extract showed statistically nonsignificant values (P > 0.05).

This indicates that the color change obtained with the use of 30% hydrogen peroxide was achieved even with the help of 10% hydrogen peroxide along with pineapple extract.

When comparing among the subgroup at all concentrations of hydrogen peroxide along with pineapple extract, the color change obtained after 20 min was significantly higher than 10 min (P < 0.05).

The results clearly indicate the significant role of vegetative enzymes in improving the efficacy of hydrogen peroxide at lower concentrations.

**DISCUSSION**

Management of discolored dentition has become a common procedure in our day-to-day practice. Discoloration of teeth can be either intrinsic or extrinsic. The management protocol of these discolorations would depend on the etiology starting from the most conservative procedure – bleaching to extensive full coverage veneer. Sometimes, patients do seek treatment just to whiten the teeth, for which bleaching becomes the primary mode of treatment.[1,2]

Lot of agents and techniques are available for bleaching, but all of them involve directly or indirectly hydrogen peroxide. The pure form of hydrogen peroxide is colorless and it is a potent oxidizing agent. Hydrogen peroxide cleaves the organic color ring molecules and makes them colorless by a simple oxidizing procedure, thus resulting in lightening of the tooth color.[3,10,11]

Commercially available bleaching agents contain a wide spectrum of the concentration of hydrogen peroxide or its precursor carbamide peroxide to suit individual clinical needs. It is obvious that higher concentration of hydrogen peroxide is always be tagged with its own side effects. The side effects would include the injuries to the hard and soft tissues of the oral cavity.[11] Hydrogen peroxide at a microscopic level causes surfaces roughness due to its action and on prolonged exposure would also destroy the organic component by disrupting the intraprismatic and interprismatic enamel.[5,12,22] Hydrogen peroxide also has the potential to penetrate deep into the dentin surface thus causing postoperative sensitivity. In addition, hydrogen peroxide causes irritation or burns to the soft tissues in the oral cavity.[15] Hence, there is always a quest to find alternatives to hydrogen peroxide. The results of various studies in the field of food chemistry have shown that certain vegetative enzymes have a good antioxidant effect. The use of these enzymes along with hydrogen peroxide has been proposed as a viable alternative for bleaching.[8] Hence, this in vitro investigation was taken up to evaluate the efficacy of use of vegetative enzymes along with hydrogen peroxide for bleaching human enamel.

In this study, vegetative enzyme extracted from pineapple was used. Pineapple contains bromelain, catalase, and polyphenol peroxidase. The pH of the extract ranges from 3 to 6.5; in this pH, the components of the extract are stable. At this pH, the extract obtained from pineapple causes disruption of adhered proteins causing stains. Bromelain present in this extract acts as a predominant oxidizing agent.[9] These enzymes are carefully extracted from pineaple by the protocols established earlier. Three different concentrations of hydrogen peroxide along with pineapple extract at two different durations of exposure were taken up for evaluation. The variables mentioned above were taken up to decipher the optimal concentration of hydrogen peroxide. The results were evaluated using a reflectance spectrophotometer.[19]

Although colorimeter is another alternative mode to evaluate the change in colors, it has its own inherent drawback of not able to detect color in the curved surface like tooth structure. And moreover, it has been established that spectrophotometer demonstrates high level of accuracy and reproducibility. The color change values obtained from the spectrophotometer were analyzed on the basis of CIE values. The use of L*a*b values were recommended by CIE. The reason being these values were used in the study because they are more close to human perception.[16,19,21]

The results of the study indicated that mean ∆E values obtained from the groups which used pineapple extract
along with hydrogen peroxide showed significantly higher lightening of tooth color when compared with group with only hydrogen peroxide. In addition, when comparing the mean ∆E values within the groups, it was found that the values were statistically nonsignificant. Furthermore, it was vivid that the longer time of exposure gave statistically significant whitening.

The results obtained in the study can be directly attributed to the role of enzyme extracts obtained from pineapple. The proteolytic enzyme bromelain has played a major role in bleaching process, by removing or disrupting the protein portion of the pellicle layer adhered to the tooth surface. The role of bromelain has been well established by the food chemistry research. The enzymes added to the experimental bleaching solution reduced the activation energy of hydrogen peroxide (75 kJ/mol) at the same time increased the rate of release of free radicals.[9,23] The results obtained in the study can be attributed to the above fact that the enzymes present in the pineapple significantly reduced the activation energy of hydrogen peroxide at the same time increased the efficacy of the rate of chemical reaction. The enzymes form a complex with hydrogen peroxide and thus improve its efficacy in bleaching, with less deleterious effects on enamel surface. Furthermore, the reaction time is hastened due to this reason. The above facts clearly explain the reason for the bleaching performance of hydrogen peroxide along with enzyme results obtained in this study. Thus, hydrogen peroxide along with pineapple extract showed promising results.[22] The sample size and the bleaching time of the specimens are the major limitations of the study.

**CONCLUSION**

Within the limitations of this study, hydrogen peroxide along with pineapple extract as a bleaching agent resulted in significant color change on stained human enamel when compared to the use of hydrogen peroxide without any additives.

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**Conflicts of interest**

There are no conflicts of interest.

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