

Effect of Bleaching with Natural Enzyme on Human Primary Teeth

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Abstract

Background

An attractive smile plays a major role in the overall perception of physical attractiveness. Since bleaching is conservative, noninvasive, and inexpensive, it is the most opted treatment protocol by the masses. Lot of agents and techniques are available for bleaching, but all of them involve directly or indirectly hydrogen peroxide. Higher concentration of hydrogen peroxide is always be tagged with its own side effects. 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.

Methods

30 human primary tooth were collected. Stained using iron supplements for 24 hrs. Divided into 2 groups: (with and without pineapple extract). Bleaching protocol followed. Colour change was evaluated using Colorimeter. The data was then collected and statistically analyzed.

Results

The analysis of results was done statistically using student's t-test. In both the groups 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 with hydrogen peroxide only ($P < 0.05$).

Conclusion

Hydrogen peroxide when used 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.

Keywords

Pineapple extract, bromelain enzyme, bleaching, hydrogen peroxide

Introduction

Esthetic problems in childhood and adolescence can have a significant effect on psychosocial development and interaction with peers.¹ Esthetic problems in childhood and adolescence can have a significant effect on psychosocial development and interaction with peers. Compromise in the esthetics, demands for dental treatment that improves the tooth color and re-establishes patient's smile. Nowadays, both children and parents place a greater value on appearance and aesthetics than earlier. Children and their parents are worried as esthetics is impaired and eventually demands for non-invasive procedure to improve the tooth color. The paradigm shift towards the non-invasive dental treatment have led to the evolution of various material and whitening techniques that are capable of re-establishing patient's smile. Since bleaching is conservative, non-invasive, and

inexpensive, it is the most opted treatment protocol by the masses.²

Various agents and techniques are used for bleaching whose primary ingredient 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.³ Although hydrogen peroxide provides better results, its side effects are inevitable. The scientific evidence also shows the change in surface texture, composition, and microhardness of enamel are reported when bleaching is done with hydrogen peroxide.^[4-6] Various attempts have been made in achieving better result of bleaching with different agents by reducing the concentration of hydrogen peroxide to reduce its side effects.

One of these efforts include the addition of vegetative enzymes as an additive to hydrogen peroxide and also lowering down its concentration to achieve the desired results. These vegetative enzymes are capable of promoting and accelerating the bleaching effect along with hydrogen peroxide.⁷

The present study aims at providing a new formulation containing enzymes obtained from the fruit "pineapple" for bleaching the primary tooth. Pineapple has a rich source of vegetative enzymes such as bromelain, catalase, and polyphenol peroxidase which are hypothesized in providing better result.^{8,9} The aim of this invitro study was to evaluate the role of pineapple natural extract as an additive to various concentration of hydrogen peroxide in bleaching artificially stained human primary teeth enamel.

Materials and Methods

Specimen Collection

For this in vitro study, thirty human primary teeth were collected. The teeth were scrutinized for

visible cracks, caries defects, and decalcifications. The defective teeth were discarded. Afterwards, the teeth were cleaned of calculus and the remaining soft tissue using an ultrasonic scaler (Satelec, India). The samples 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 chopped 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

The specimens were kept in a container having iron supplement in syrup form for 24 hours.

The specimens are then randomly divided into 2 groups:-

Group I (n=15)- 30% hydrogen peroxide

Group II (n=15)- 20% hydrogen peroxide

The roots of all teeth were cut at CEJ. The crowns were sectioned equally into mesial and distal

halves labio-palatally. They were mounted exposing the labial surface, using self-cure resin. 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 consists of 1 ml of pineapple extract + 1 ml of phosphate buffered solution + 28 ml of hydrogen peroxide. They were proportioned using pipette and stirred in a test tube.

After bleaching, the teeth were thoroughly rinsed and stored in artificial saliva at 37°C for 24 h.

Testing Methodology

The testing was done with colorimeter over a white background, which recorded color variables L*, a*, b* in accordance to CIE L*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 ΔE values' standard deviation

GROUP I		GROUP II	
30% Hydrogen Peroxide	30%Hydrogen Peroxide + extract	20% Hydrogen Peroxide	20% Hydrogen Peroxide + extract
24.08±1.32	32.51±0.62	23.81±1.23	29.95±0.6

In both the groups 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 with hydrogen peroxide only (P < 0.05).

The color change obtained with the use of 30% hydrogen peroxide was achieved even with the help of 20% hydrogen peroxide along with pineapple extract. The results clearly marks the significant role of vegetative enzymes in improving the efficacy of hydrogen peroxide at lower concentrations.

Discussion

Lot of agents and techniques are available for bleaching, but all of them involve hydrogen peroxide directly or indirectly. The pure form of hydrogen peroxide is a potent oxidizing agent and is colorless. The lightening of tooth color occurs as hydrogen peroxide cleaves the organic color ring molecules and makes them colorless by a simple oxidizing procedure.^{3,10,11}

Wide variety of bleaching agents are available commercially containing various concentration of hydrogen peroxide or its precursor carbamide peroxide to match individual clinical needs. It is obvious that higher concentration of hydrogen peroxide always come with its own side effects. The side effects eventually includes the harm to the hard and soft tissues of the oral cavity.¹¹ Hydrogen peroxide at a microscopic level causes surface roughness due to its action and on prolonged exposure it can also disrupt the intraprismatic and interprismatic enamel by damaging the organic component.^{5,12,13}

Hydrogen peroxide can cause postoperative sensitivity by penetrating deep into the dentinal surface. In addition, hydrogen peroxide causes irritation or burns to the soft tissues in the oral cavity.¹³ Hence, there is always a quest to find alternatives to hydrogen peroxide. Various studies in field of food chemistry has shown that certain vegetative enzymes have good antioxidant effect. Using such enzymes along with hydrogen peroxide has been proposed as an alternative

for bleaching.⁸ Hence, this *in vitro* study was taken up to evaluate the efficacy of vegetative enzyme when used along with hydrogen peroxide for bleaching primary tooth human enamel.

In present investigation, vegetative enzymes were extracted from pineapple. Pineapple contains bromelain, catalase, and polyphenol peroxidase. The pH of this pineapple extract ranges from 3 to 6.5; the pH, at which the components of the extract are stable. At this pH, the extract gained from pineapple cause disruption of adhered stain causing proteins. Bromelain present in this extract acts as a predominant oxidizing agent.⁹ These enzymes are carefully extracted from pineapple by the protocols established earlier.

This study was conducted using colorimeter. using spectrophotometer would be a good choice as it demonstrates high level of accuracy and reproducibility.

The results indicate that mean ΔE values obtained from the group which used pineapple extract along with hydrogen peroxide showed significantly better lightening of tooth color when compared with group with only hydrogen peroxide.

The results obtained can be directly attributed to the role of vegetative enzymes which are extracted 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 food chemistry research has well established the role of bromelain. 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.^{9,14} The results obtained in the study can be attributed to the fact that the enzymes present in the pineapple significantly reduced the activation energy of hydrogen peroxide and at the same time increased the

efficacy of the rate of chemical reaction. The enzyme improves the efficacy of hydrogen peroxide in bleaching by forming a complex, with less deleterious effects on enamel surface. Subsequently, the reaction time is hastened due to this reason. The above facts are strong enough to explain the reason for better bleaching performance of hydrogen peroxide when used along with enzyme. Thus, hydrogen peroxide along with pineapple extract showed promising results.¹³ The sample size and the bleaching time of the specimens are the major limitations of the study.

Conclusion

Within the limitations of this study, pineapple extract when used along with hydrogen peroxide results in significant color change on stained human enamel compared to using hydrogen peroxide without any additives.

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