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Effect of Nanohydroxyapatite Incorporation in Hydrogen Peroxide Bleaching Agent on Minerals Content of Dental Enamel

Asraa Sabah Abtan [(1) *]
Nibal Mohammed Hoobi [(2)]

[(1,2)] Department of Pediatric and Preventive Dentistry, College of Dentistry, University of Baghdad Iraq.

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***Corresponding Author:**
Email:
asraasabah90@gmail.com

Department of Pediatric and Preventive Dentistry,
College of Dentistry,
University of Baghdad, Iraq.

Abstract

Background: Current study was assessed to evaluate the effect of nanoydroxyapatite (nHAp) incorporation in hydrogen peroxide (HP) bleaching agent on selected minerals content of dental enamel. Material and Methods: 30 sound upper first premolar teeth were used in this study. Enamel blocks (7mm× 5mm×3mm) were obtained from the middle third of buccal surfaces of the teeth. Each dental block was embedded in self-cure acrylic resin with exposed exterior enamel surface. Enamel samples were divided into three groups (n=10): deionized water (DW) as control group, HP bleached group, and hydrogen peroxide modified by nanohydroxyapatite (HP-nHAp) bleached group. Energy Dispersive X-Ray Spectroscopy (EDS) was used to evaluate the elemental composition changes of each sample before and after treatment. Result: Findings of the current study revealed reduction of each of enamel calcium (Ca) and phosphorous (P) minerals content after treatment compared with baseline data for all groups, with statistically significant difference for HP group only. The percentage of Ca and P weight loss of enamel subjected to HP bleaching was the highest, followed by HP-nHAp treated group, while the control group was the least one with statistically significant difference between each of (HP and HP-nHAp) groups, and (HP and control) groups. No significant reductions were observed after treatment in Ca/P ratio in all groups. Conclusion: Adding of nHAp material to HP bleaching agent could decrease its adverse effects on dental enamel surface.

Introduction:

The increasing demand for an esthetic dentistry particularly teeth color is mainly due to patients' growing concerns about their appearance and any staining or discolorations can have a significant effect on their quality of life. Tooth discolorations are categorized as intrinsic or extrinsic discolorations and they differs in their etiology, severity, appearance, localization, and adherence to tooth structure[1]. Dental bleaching is considered to be the most safe, conservative, and effective method used to treat discolored teeth [2]. Many different bleaching systems have been introduced in the market and they commonly divided as: dentist supervised at home bleaching, in office bleaching, and over the counter bleaching products. High concentrations of HP (30-40%) are commonly used during in office bleaching [3].
Bleaching agents mechanism is complex and dynamic which involves penetrating of HP and diffusion into enamel and dentin and releasing free radicals that oxidize chromophores, which are electron rich unsaturated molecules that absorbs specific wavelengths of visible light. When the chromophores attacked by the free radicals, resulting in the splinting of double bonds and rendering them colorless, presenting a lighter aspect of the tooth [4].
Although HP has been shown to be effective, there are still significant concerns about its negative impact on the dental enamel. Several studies have shown that the bleaching agents can cause structural, mechanical, and chemical changes in enamel such as increased roughness, decreased microhardness, an alteration in enamel minerals content [3,5-8]. In addition it was reported that higher concentration of HP bleaching product could induce further damage to the pulp [9].
Various remineralizing compounds can be utilized to reduce the detrimental impacts of bleaching procedures on dental enamel such as calcium, fluoride, and amorphous calcium phosphate. These ingredients are incorporated in bleaching gels in order to prevent the demineralization of the enamel while
bleaching, as well as to decreases or avoid the dental sensitivity, which many patients have complained during and/or following bleaching procedures [1].
The clinical application of nHAp represents one of the last frontiers to overcoming the demineralization. It has a noticeable re-mineralizing effects on initial lesions of dental enamel, it provide a protection against caries and dental erosion [10]. nHAp have a morphological and structural similarity to tooth hydroxyapatite crystals with superior properties such as higher surface energy, better biocompatibility and increased solubility compared to hydroxyapatite [11]. Studies have proven its potential remineralization of dental enamel [12-14]. It is also found that the nHAp has ability to preserve the enamel morphology and preventing minerals loss after exposure to an acidic beverage [15]. Energy dispersive X-ray spectroscopy (EDS) is an analytical technique used for the elemental analysis or chemical characterization of a sample. The EDS analysis can be used to determine the elemental composition of individual points or to map out the lateral distribution of elements from the imaged area. EDS systems are generally attached to an scanning electron microscopy and it is based on the measurement of the energy of characteristic X-ray emissions of excited samples[16].
Thus, the present study was aimed to assess the effect of nHAP incorporating in 40% HP gel on enamel minerals content.

Materials and Method

Sample size

It was been calculated by using G power 3.0.10 (Program written by Franz-Faul, Universitatit Kiel, Germany) With power of study=80%, alpha error of probability=0.05 two sided, effect size of F is 0.60 (Large effect size), with three groups, with all these condition the definitely sample size is 30 samples (10 samples for each group) [17]

Preparations of enamel samples

Thirty maxillary premolar teeth were extracted for orthodontic treatment purpose, cleaned and rainsed using tap water, then polished with a non fluoridated pumice slurry. Teeth were examined using a magnifying lens (10X), any tooth had a visible cracks, caries, or structural enamel defects was discarded. The teeth are subsequently immersed in 0.1% thymol solution which acts as an antimicrobial agent to prevent bacterial growth until use. Enamel samples were prepared by cutting the teeth horizontally in the cervical area to separate crowns from roots, then the crown of the each tooth was longitudinally sectioned in mesio-distal direction to separate buccal and lingual surfaces. Enamel blocks of uniform dimensions 7×5mm and 3mm thickness were prepared from the middle third of the buccal surface of each tooth using a double-sided diamond disk (Dental Lab Diamond disc, Airgoesin, Brazil) and micro-motor handpiece with water cooling, and they were calibrated using an electronic digital caliper to obtain correct measurements. Each enamel block was individually inserted in self-curing acrylic resin (DMP Ltd, E.U) with exposed buccal enamel surfaces (Figure1). All the samples then stored individually in plastic container containing DW ready for baseline measurements.

Sample grouping

Enamel samples were divided in to the three groups according to the bleaching technique as follow :

I. HP group: the samples were treated with 40% HP bleaching gel.
II. HP-nHAp group: the samples were treated with 40% HP bleaching gel modified by incorporation of nHAp powder.
III. Control group: the samples were not undergone any treatment and only stored in DW during experimental period.

All the enamel samples stored in DW throughout the experiment as storage media except during bleaching procedure [18].

Minerals composition analysis analysis before bleaching procedure

The proportions of Ca and P wt% in each sample were studied using energy-dispersive X-ray spectroscopy (Axia-ChemiSEM, Thermo Fisher Scientific, USA). The specimens were positioned on a metal stump with their labial surfaces facing upwards and then analyzed (Figure 2). The specified operating conditions were as follows: a working distance of 10 mm, an accelerating voltage of 20 kV, an acquisition time ranging from 10 to 30 , and a magnification ×2500. Ca and P contents were determined using a standard mode, with three peak areas analyzed for each sample. The average percentages of Ca and P were computed for each sample, followed by the calculation of the Ca/P ratios.

Bleaching procedure:

Hydrogen peroxide bleaching technique

The bleaching procedure was performed by using chemical bleaching system (40% HP gel Opalescence Boost, UT, USA) according to the manufacturer's instructions, the product was activated by syringe-to-syringe mixing. About 1 mm thick layer of HP gel was applied to enamel samples and left for 20 min. Then the gel was rinsed with DW for 10 s. Gel application was repeated two more times in the same session for each sample to have a total 60 minutes of application. The bleaching procedure was repeated after one week to obtain the total of two bleaching sessions.

Hydrogen peroxide modified by nanohydroxyapatite bleaching technique

For preparation of HP-nHAp mixture, 2 g of nHAp powder (Sigma-Aldrich, USA; particle size < 200 nm) was mixed with 1 ml of DW. After that, they were mixed with 1 ml of 40% HP [19] (Figure 3). The HP-nHAp mixture was applied to enamel surface for 20 min then rained with DW for 10 s. This procedure was repeated two more times to have a total of 60 minutes of application. The bleaching procedure was repeated after one week to obtain the total of two bleaching sessions.

Minerals composition analysis after bleaching

Ca, P, and Ca/P ratio contents were measured after bleaching procedure with EDS as the same way that measured before the bleaching.

Statistical analysis

The obtained data was statistically analyzed by using Statistical Package for Social Science (SPSS) version 22. The normality of data was tested by Shapiro-Wilk test. One way ANOVA and Tukey post hoc multiple comparisons tests were used to compare among groups. Additionally, a paired *t*-test was analyzed the enamel elemental difference between before and after treatment. The level of significance for all statistical tests was $p<0.05$.

Results

Calcium mineral content

The results of dental minerals concentration and distribution per sample from EDS analysis appears as shown in Table (1), Figure (4), and Figure (5). Mean values, standard deviations, and statistical difference of Ca wt% at the baseline and after treatment of three groups were shown in Table (2). There was a statistically significant reduction of Ca wt% in HP group, while it was not significantly reduced in HP-nHAp and control groups. Figure (6) shows the mean values of the percentage of Ca wt% loss after bleaching in three groups and the highest loss was in HP group compared with other two groups. The result from multiple pairwise comparisons of Ca wt% loss among three groups is illustrated in Table (3). A statistical significant difference was found between (HP and control) and (HP and HP-nHAp) groups, while there was no significant difference between (HP-nHAp and control) groups.

Phosphorous mineral content

Table (4) shows the mean values and statistical difference of P wt% at baseline and after treatment of three groups. The result demonstrated that there was a statistically significant reduction of P wt% in HP group, while it was not significantly reduced in HP-nHAp and control groups. Figure (7) shows the mean values of the percentage of P wt% loss after treatment in three groups and the highest loss was in HP group compared with other two groups Multiple comparisons of P wt% loss among three groups have been shown in Table (5). A statistical significant difference was found between (HP and control) groups and (HP and HP-nHAp), while it was not significant difference between (HP-nHAp and control) groups.

Calcium phosphorus ratio measurement

Table (6) illustrates the mean values and statistical difference of Ca/P ratio at baseline and after treatment of three groups. There was a statistically not significant reduction in Ca/P ratio for all groups after the treatment. Descriptive statistics and statistical differences of percentage of Ca/P ratio reduction after treatment have been shown in Table (7). The highest reduction has been found in HP group followed by HP-nHAp group, while the least percentage of reduction was recorded for control group with no statistical significance difference among three groups.

Discussion

Bleaching has been accepted as a highly effective technique for treating tooth discolouration and is regarded as a conservative method for obtaining aesthetic or cosmetic benefits. Tooth whitening treatment is gaining popularity, multiple bleaching agents available in the market with different techniques [20]. The bleaching material is based of several peroxide compounds, which can potentially have negative effects.

nHAp material was used in the current study due to its biocompatibility and having a chemical and structural resemblance to natural bone and tooth minerals, as well as its lack of irritant properties. The nHAp exhibits a high affinity for demineralized surfaces because it can effectively enter the pores of enamel and serve as a template during the precipitation process. This promotes the growth and integrity of crystals[21].

Enamel samples were stored in DW between bleaching sessions and not stored in an artificially created saliva solution because artificial saliva contains Ca and P, allowing enamel remineralization that reverses the effect produced by the bleaching procedure and act as a confounding factor that masks the actual effects of bleaching on minerals contents of enamel [22,23].

Dental enamel is hardest tissue of human body, being highly mineralized containing 96% inorganic, 2% organic, and 2% water components. Hydroxyapatite crystal in the form of a crystalline lattice is the major constituent and represents (92-98)% of overall inorganic matter [5]. Enamel solubility is strongly influenced by the pH of the surrounding material, as well as the concentrations of Ca, P, and to a minor level fluoride ions [24,25]. Ca and P, are essential components of enamel and play a crucial role in its structure. A decrease in the levels of these two elements can cause irreversible alterations and preventing the re-mineralization process from occurring[18].

In this study, enamel minerals were evaluated using EDS, as it is a popular, precise and nondestructive technique for studying the enamel minerals component [26]. In the present study, the findings revealed a statistically significant reduction in the Ca and P wt% after treatment for group treated with HP alone. Ca and P ions are lost during bleaching applications due to the dissolution of hydroxyapatite crystals [27], the interaction between acidic bleaching agent and hydroxyapatite results in the following reaction [28] :

$$\mathbf{Ca_{10}(PO_4)_6(OH)2+8H+\rightarrow 10Ca^{2+}+6HPO_4{}^{2-}+2H_2O}$$

The unstable and nonspecific oxygen free radicals that released by HP gel not react only with the chromophores molecules but also react with the organic structures of dental enamel causing oxidation of the proteins in its compositions. The degradation of these substances enhancing removal of related minerals elements [29]. In the decomposition process, HP free radicals can also interact with inorganic elements leading to gradual dissolution of the enamel surface by removal of mineral elements, affecting enamel integrity and promoting carbonate loss which decreases minerals content of the enamel [30]. After the first and second peroxide treatments, the majority of the Ca was lost from the hydroxyapatite due to its weaker bound [31] . According to Wang et al. [32], the release of Ca from the enamel apatite may occur via atomic diffusion through the apatite channels along the crystallographic c-axis and the inter-crystallites and inter-rod special voids with openings on the surface. This results was in agreement with Basheer et al. [2], who reported a significant Ca and P minerals loss after dental bleaching. In contrast to the study done by Kheradmand et al. [33] who concluded that HP bleaching material may not induce loss of superficial enamel mineral content.

On other hand, nHAp played an important role to preserving the enamel's structure throughout the bleaching procedure. Indeed, nHAp is an alkaline salt that raises the pH of HP solution from about 3.2 to approximately 5.4, hence reducing its acidity[8]. Furthermore, the nHAp particles have the ability to adhere evenly to the enamel surface, forming a protective coating that reduces the direct contact between HP and the enamel surface [20]. Moreover, nHAp can help to remineralize tooth surface by delivering Ca and P to the area of demineralized enamel [34]. This finding came in agreement with with Basheer et al. [2] and Kheradmand et al.[33] who concluded that "the addition of nHAp to the bleaching agent may improve the mineral content (Ca and P) of superficial enamel". Changes in the Ca/P ratio indicate alterations in the inorganic components of hydroxyapatite [35]. It is well known that the Ca/P ratio can be altered by the high HP concentrations [36]. The result of this study found that there was a reduction in the Ca/P ratio after treatment for all groups. However, there was no statistically significant difference for all of them. This lack of significant effect of the bleaching agent on the Ca/P ratio is also documented by Orilisi et al. [18].

Conclusion

From this in vitro study, it can be concluded that the incorporation of nHAp in HP bleaching agent could significantly reducing the enamel minerals loss and thereby minimizing its detrimental effect on tooth surface.

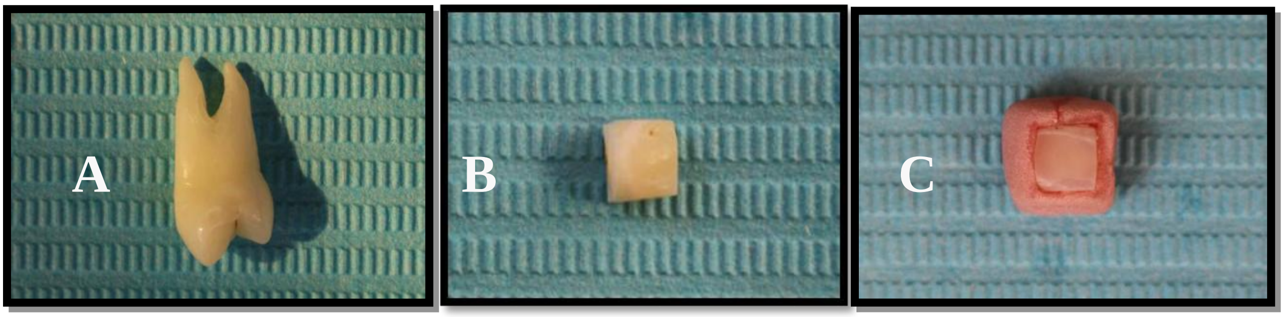

Figure 1: Enamel sample preparation. A: Sound extracted maxillary first premolar. B: Enamel block. C: Enamel block embedded in self-curing acrylic resin.

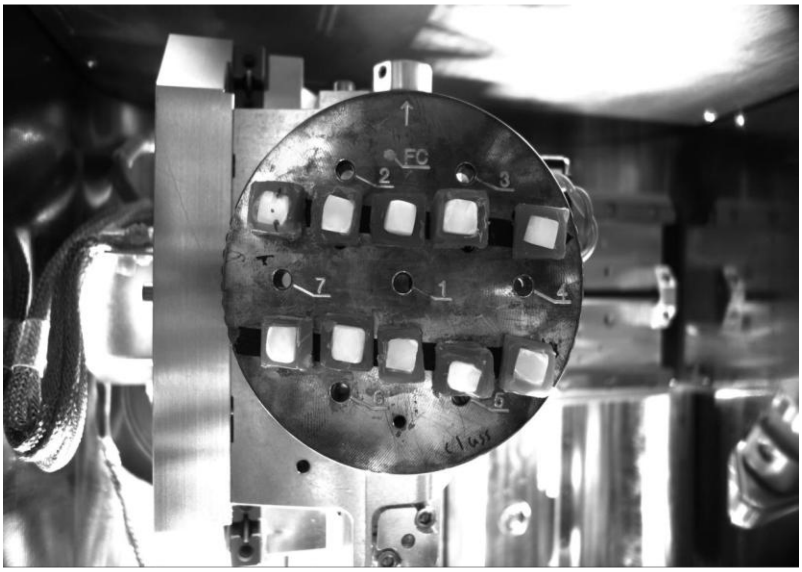

Figure 2: Enamel samples arranged over a metal stump inside Energy-dispersive X-ray spectroscopy

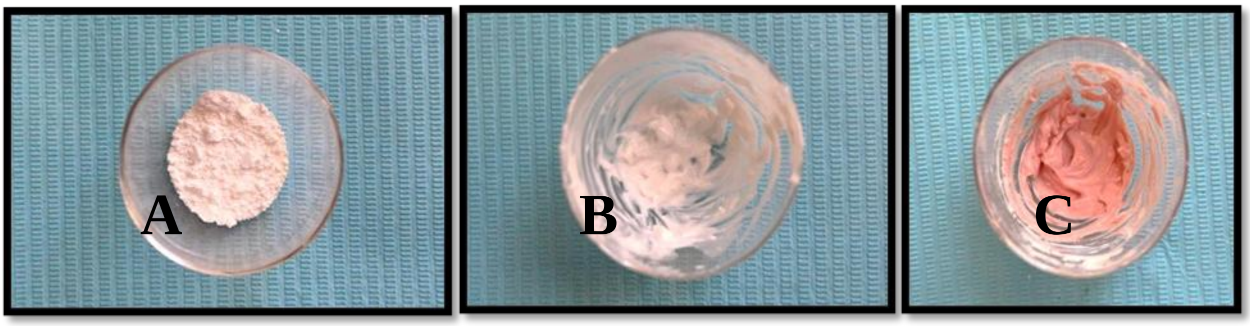

Figure 3: Preparation of hydrogen peroxide nanohydroxyapatite mixture. A: nanohydroxyapatite powder. B: nanohydroxyapatite mixed with deionized water. C: nanohydroxyapatite mixed with hydrogen peroxide.

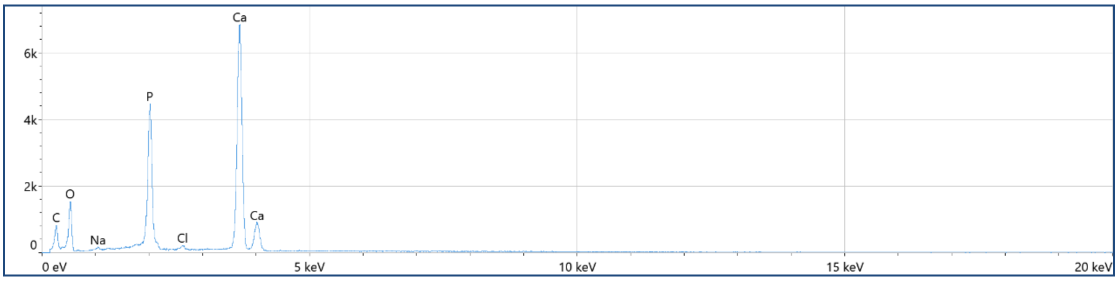

Figure 4: Energy dispersive X-ray spectroscopy shows the elemental concentrations of one sample.

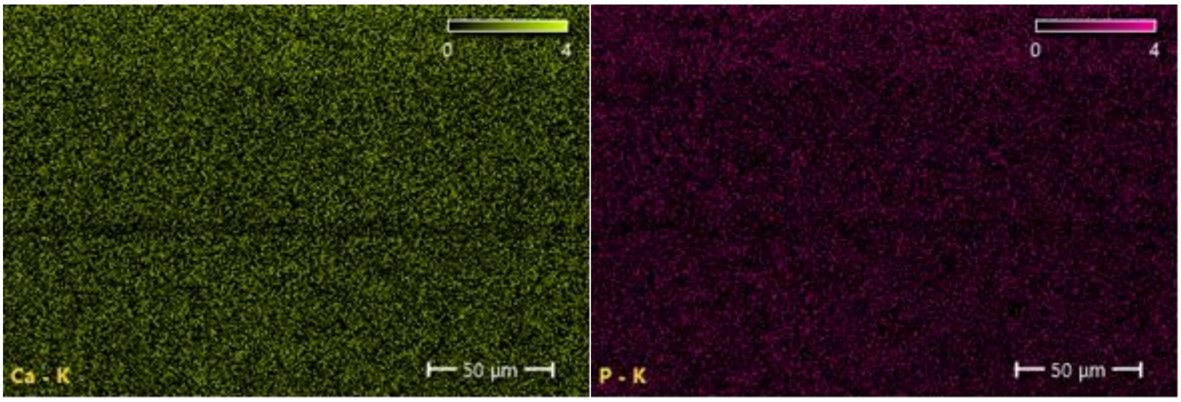

Figure 5: Energy dispersive X-ray spectroscopy shows the map of calcium and phosphorous minerals distribution of one sample.

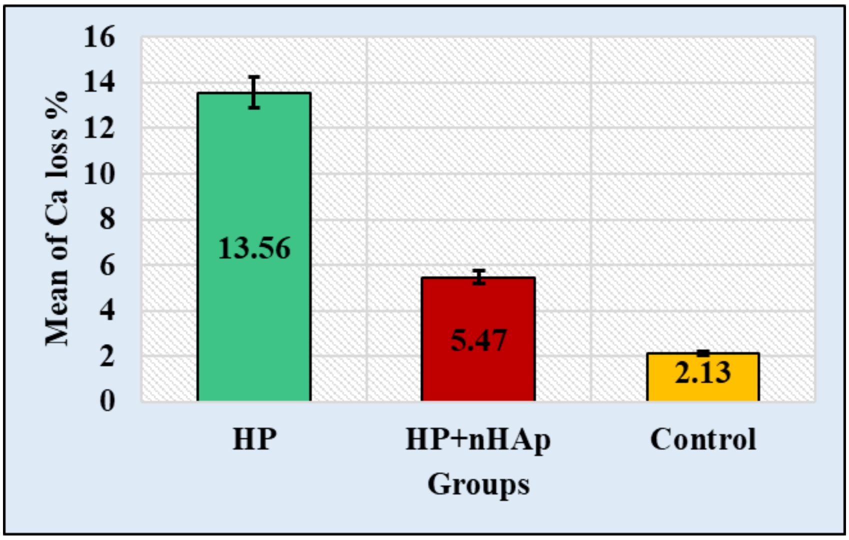

Figure 6: Mean of calcium weight percentage loss after treatment for all groups.

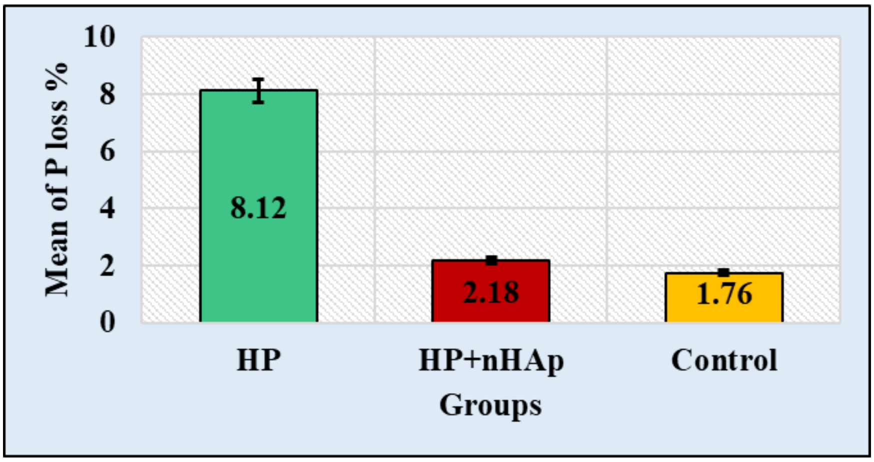

Figure 7: Mean of phosphorous weight percentage loss after treatment for all groups.

Table 1: Energy dispersive X-ray spectroscopy shows the elemental concentrations of one sample.

Weight % Error	Weight %	Atomic % Error	Atomic %	Element
0.3	14.3	0.6	24.0	C
0.5	40.6	0.7	51.1	O
0.1	0.6	0.1	0.5	Na
0.1	14.0	0.1	9.1	P
0.0	0.4	0.0	0.3	Cl
0.2	30.1	0.1	15.1	Ca

Table 2: Calcium weight percentage before and after treatment (mean±SD)and statistical difference among various groups.

Groups	Baseline Mean ± SD	After treatment Mean ±SD	Paired T test	P value
Hydrogen peroxide	35.71 ± 3.58	30.76 ± 2.60	7.65	<0.001*
Hydrogen peroxide + nanohydroxyapatite	35.28 ± 2.79	33.31 ± 2.41	1.98	0.051
Control	34.54 ± 3.16	33.79 ± 3.01	1.67	0.072

Table 3: Multiple comparison of calcium weight percentage loss of different groups.

Groups		Mean difference (I-J)	P value
Hydrogen peroxide	Hydrogen peroxide + nanohydroxyapatite	8.087	0.001*
	Control	11.427	0.001*
Hydrogen peroxide + nanohydroxyapatite	Control	3.340	0.056

Table 4: Phosphorous weight percentage before and after treatment (Mean ± SD) and statistical difference among various groups.

Groups	Baseline Mean ± SD	After treatment Mean ± SD	Paired T test	P value
Hydrogen peroxide	14.12 ± 1.06	12.97 ± 1.03	8.77	0.001*
Hydrogen peroxide + nanohydroxyapatite	14.58 ± 0.39	14.26 ± 0.40	2.94	0.052
Control	14.30 ± 0.63	14.03 ± 0.38	1.50	0.167

Table 5: Multiple comparison of phosphorous weight percentage loss of different groups.

Groups		Mean difference (I-J)	P value
Hydrogen peroxide	Hydrogen peroxide + nanohydroxyapatite	5.94	0.001*
	Control	6.36	0.001*
Hydrogen peroxide + nanohydroxyapatite	Control	4.21	0.752

Table 6: Calcium phosphorus ratio before and after treatment (Mean ± SD) and statistical difference among various groups.

Groups	Baseline Mean ±SD	After treatment Mean ±SD	Paired T test	P value
Hydrogen peroxide	2.53 ± 0.23	2.38 ± 0.24	2.47	0.075
Hydrogen peroxide + nanohydroxyapatite	2.42 ± 0.17	2.33 ± 0.14	2.38	0.081
Control	2.41 ± 0.16	2.40 ± 0.21	0.213	0.836

Table 7: Percentage of calcium phosphorus ratio reduction after treatment (Mean ± SD) and statistical difference among various groups.

Groups	Mean ± SD	F test	P value
Hydrogen peroxide	5.91 ± 2.25	2.81	0.062
Hydrogen peroxide + nanohydroxyapatite	3.40 ± 1.42		
Control	3.21 ± 1.03		

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