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COMPARATIVE STUDY ON THE EFFECT OF CALCIUM CARBONATE AND COVARINE ON THE ENAMEL STRUCTURE OF HUMAN TEETH (IN VITRO STUDY)

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ABSTRACT

Toothpaste is a major preventive to enamel surface calcium carbonate as one of abrasives in bleaching toothpastes has an antibacterial effect. A new optical technique is to use covarine blue in bleaching toothpastes.

Objectives: Comparison between calcium carbonate and covarine blue toothpastes on enamel structure, minerals content and microhardness.

Methodology: 48 human premolars divided into three groups: Group I :16 premolars preserved in distilled water. Group II :16 premolars brushed by calcium carbonate gel. Group III :16 premolars brushed by covarine gel. Buccal surface of all premolars was brushed electrically and each group was subdivided into 2 subgroups, one examined after 3 weeks and the other after 7 weeks. Enamel was then assessed by: scanning electron microscope (SEM), energy dispersive x-ray analyzer (EDXA) and microhardness tester.

Results: SEM: Obvious precipitation of calcium particles on enamel surface in calcium carbonate group. Covarine blue group showed almost the same findings as control group except for few cracks. EDXA: After 3 weeks the highest C and P wt. % was recorded in calcuim carbonate group and covarine blue group respectively, while there was no difference in Ca % in both groups. After 7 weeks P % was the highest in calcium carbonate group. There was no statistical significant difference between all groups regarding weight % of calcium and carbon.

Microhardness: Calcium carbonate group represented an increase in the difference of microhardness from 3 to 7 weeks.

Conclusions: Calcium carbonate toothpaste caused precipitation of calcium particles on tooth surface and increase the microhardness of enamel in comparison to covarine blue.

KEYWORDS: Enamel; Calcium carbonate; Covarine blue; Bleaching toothpaste

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INTRODUCTION

Enamel is the hardest substance within the human body and is the wear-resistant outer layer of the crown. It forms an insulating barrier that protecting tooth from physical, thermal, and chemical forces which may be injurious to the important tissue within the underlying pulp. The optical properties of enamel have been derived from its structure, composition, biological process defects, or environmental influences on enamel structure [1].

It is feasible to make an effort to improve the shade of teeth, utilizing a variety of techniques, including expert scaling and prophylaxis carried out in a dental office, tooth augmentation the usage of laminate veneers, crowns, and tooth whitening. Tooth-whitening formulas function by one of two approaches, either by getting rid of extrinsic tooth discoloration or through tooth bleaching [2].

Calcium carbonate is frequently included in the rocks, chalk, limestone, and marble as its most prevalent natural forms. It is a part of tougher organic materials like oyster and clam shells and eggshells. It serves as a dietary calcium source in some soy milk and almond milk products. Additionally, calcium carbonate is frequently utilized as a nutritional supplement or antacid for the stomach. It has a mildly gritty flavor and is a fine, white, odorless powder [3]. Calcium carbonate has a mild abrasive property which helps to safely remove plaque when brushing and gently polishes away surface stains [4].

Small particles of calcium carbonate (0.7-10 um) work as abrasives on enamel surfaces. In the 1850s, calcium carbonate was first widely used as an abrasive in toothpaste formulas [5]. Ground calcium carbonate and precipitated calcium carbonate, with a ratio of up to 55%, are the most common ore minerals utilized in toothpaste abrasives [6].

The Covarine products contains highly pure organic pigment dispersions for coloring personal care products like toothpaste and soap. These products are highly pigmented, extremely stable, and consistent from batch to batch. Blue die is added to covarine as it is the opposite color of yellow [7].

Covarine is one of the optical whitening toothpastes that acting by deposition of a blue covering thin film onto the enamel surface. Depending on the standard and thickness of the film deposited, it alters the natural colors of teeth from yellowish-brown to an additional pleasing and aesthetic white-bluish color [8].

So, this study aimed to compare the effect of calcium carbonate, and covarine blue on enamel surface to detect surface morphological changes, to assess changes in calcium, phosphorous and carbon ions and to evaluate the hardness of enamel.

MATERIALS AND METHODS:

Research Ethics Committee of Faculty of Dentistry, Ain Shams University, Cairo, Egypt, exempted this study protocol.

Materials:

Teeth collection:

Forty eight extracted human sound first premolars were collected from healthy patients undergoing extraction at the surgery clinic at the Faculty of Dentistry Ain Shams University. Informed consent was obtained from patients prior to extraction.

Tooth brush:

Wanhengda electrical tooth brush was used with frequency 200 hertz (Dongguan Wanhengda Dissipation Technology Co., Ltd.).

Gel material:

Active agent materials (calcium carbonate and covarine blue) were prepared in Nano gate laboratory at Mokattam district, Cairo, Egypt.

Methods:

Gel preparation:

To prepare 100 ml of calcium carbonate gel 30% weight / volume (w/v) and 10 ml of blue covarine gel 20%w/v, 30 gm of calcium carbonate powder and 2 gm of dye powder weighed out on a sensitive balance (WTC 200, RADWAG®, Poland) and dissolved in 100 ml of deionized water with stirring by using hot plate and stirrer (MSH-20A, wetige®, Germany) for 30 min then, 6gm of carboxymethyl cellulose (Loba CHIME, India) was sprinkled gently and gradually over the solution under mild temperature with vigorous stirring to get homogenous paste [9].

Teeth grouping:

The collected premolars were brushed gently to get rid of any residual debris and were equally divided into three groups as follows:

a- Group I :16 premolars were preserved in distilled water.

b- Group II :16 premolars were brushed for one minute by calcium carbonate gel 10 ml to 30 ml distilled water day and night at an interval of 12 hours.

c- Group III :16 premolars were brushed for one minute by covarine gel 10 ml to 30 ml distilled water day and night at an interval of 12 hours.

During the interval time the teeth in each group were placed in distilled water till the end of experimental period [10].

Each group was further subdivided equally into 2 subgroups (n=8) as follows:

- **Subgroup A** → in which premolars were examined after 3 weeks.
- **Subgroup B** →in which premolars were examined after 7 weeks.

Teeth brushing:

Teeth were brushed using electrical tooth brush on standard speed for one minute to save stable brushing system and power. Active agent gel was applied on buccal surface at the middle third as a solution of distilled water and active agent gel with ratio 3:1 (distilled water: active agent gel) [9].

Assessment of enamel:

After the end of experiment the enamel of all teeth in each group was assessed by: scanning electron microscope (SEM), energy dispersive x-ray analyzer (EDXA) and microhardness tester.

Scanning electron microscope and EDXA:

Five teeth from each group were cleaned for three minutes under running water before being blotted dry outside. Then, using adhesive vinyl glue to fix the tooth on the SEM holder. Midpoint of middle third of the buccal surface's enamel was modified to measure for surface calcium (Ca), phosphorous (P) and carbon (C) ions weight percentage utilizing the linked FEI/ Inspect scanning electron microscope using a SEM-EDXA unit, an energy dispersive X-ray analyzer Laboratory of desert research center, Cairo, Egypt. In order to scan, the collected teeth were examined using an electron microscope employing the secondary electron LFD detector and being tested at 30 kV under (X 1000) and (X 5000) magnification. Surface weights Ca, P, and C Utilizing energy dispersive X-ray analysis, % were determined [11].

Microhardness tester:

This was performed on three teeth from each group using Tukon 1102 Wilson microhardness tester Buehler Germany. In the Vickers test, the load (100gm) was applied smoothly, without impact, forcing the indenter into the test specimen. The indenter was held in place for 10 seconds. The physical quality of the indenter and the accuracy of the applied load must be controlled in order to get the correct results. After the load was removed, the indentation was focused with the magnifying eye piece and the two impression diagonals were measured, usually to the nearest 0.1-µm with a micrometer, and averaged. The Vickers hardness (HV) was calculated using HV = $1854.4L/d^2$

Where the load L is in gf (gram force) and the average diagonal d was in µm (this produces hardness number units of gf/µm2. in our study the numbers were reported without indication of the units [12].

Statistical analysis:

Recorded data from EDXA analysis and microhardness test were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were presented as mean± standard deviation. Also, qualitative variables were presented as number and percentages. These tests were done by independent-samples t-test of significance was used when comparing between two means, one-way analysis of variance (ANOVA) when comparing between more than two means, Post Hoc test: Tukey's test was used for multiple comparisons between different variables and the confidence interval was set to 95% and the margin of error accepted was set to 5%. So, probability value (P-value) was considered highly significant at <0.001, significant at <0.05 and non-significant at >0.05.

RESULTS:

Scanning electron microscope results:

Subgroup A:

Control subgroup:

The enamel surface appeared smooth with some scratches. Multiple enamel pores were detected (figure 1a). With a higher magnification the enamel pores showed variable diameter, some appeared small while others showed large diameter (figure 1b).

Calcium carbonate subgroup:

Rough enamel surface with multiple enamel pores was noticed. Obvious precipitation of calcium particles on the surface was seen (figure 1c). The calcium particles appeared with different sizes (figure 1d).

Covarine blue subgroup:

Enamel surface showed multiple enamel pores and perikymata. The enamel surface appeared relatively smooth with multiple scratches (figure 1e). Enamel rods showed fish scale appearance (figure 1f).

Subgroup B:

Control subgroup:

Enamel surface appeared smooth with some scratches. Multiple enamel pores and parallel ridges "perikymata" were detected (figure 2a). Fish scale enamel prisms were noticed (figure 2b).

Calcium carbonate subgroup:

Most of enamel surface was covered by calcium precipitation, some deposits were seen in some area and enamel pores of apparently large sizes were noticed (figure 2c). Detectable calcium particles with large size were observed (figure 2d).

Covarine blue subgroup:

Fish scale enamel prisms were observed. Smooth enamel surface with some scratches were seen. Multiple enamel pores as well as cracks on the enamel surfaces were noticed (figure 2e). With a higher magnification, the enamel prisms showed fish scale shape and some scratches (figure 2f).

EDXA results:

Subgroup A:

Calcium carbonate subgroup showed the highest mean weight % of carbon followed by covarine blue subgroup. The control subgroup showed the lowest mean with no statistical significant difference between covarine and control subgroups. About P, the highest mean weight % was in control subgroup,

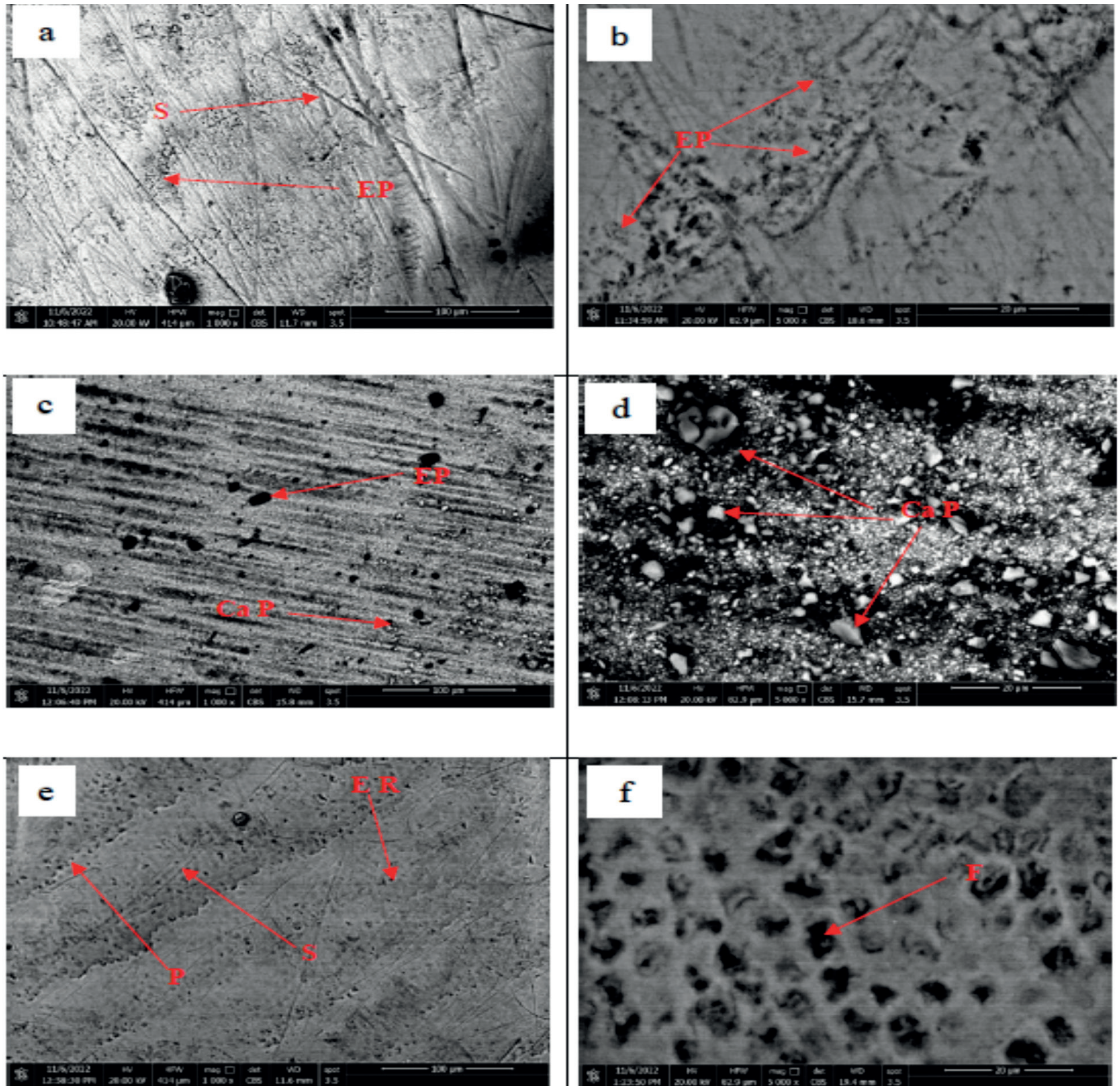


Fig. (1) Photomicrographs of enamel surface showing: (a)-Control subgroup A: scratches and enamel pores. (b)- Control subgroup A: scratches, small diameter enamel pores and large diameter enamel pores. (c)- Calcium carbonate subgroup A: calcium particles and enamel pores. (d)- Calcium carbonate subgroup A: calcium particles. (e)- Covarine blue subgroup A: scratches, enamel rod end and perikymata. (f)- Covarine blue subgroup A: fish scales prisms appearance. S, Scratches; E P, Enamel pores; Ca P, Calcium particles; E R, Enamel rod end; P, Perikymata; F, Fish scale appearance (SEM, Original magnification a, c &e x1000, b, d & f x5000).

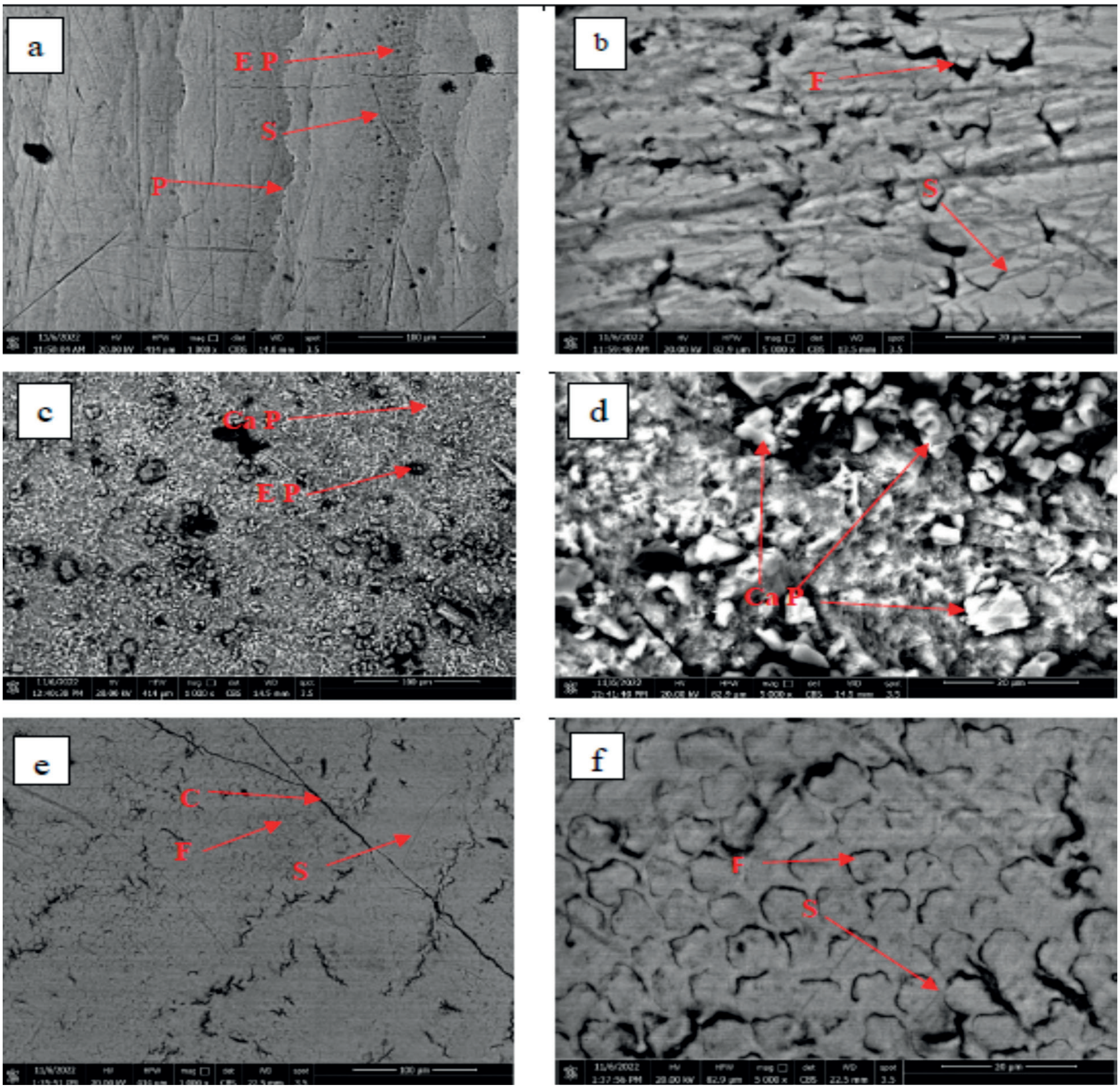


Fig. (2) Photomicrographs of enamel surface showing: (a)-Control subgroup B: scratches, enamel pores and perikymata (b)-Control subgroup B: scratches and fish scales prisms appearance. (c)- Calcium carbonate subgroup B: calcium particles and enamel pores. (d)- Calcium carbonate subgroup B: calcium particles. (e)- Covarine blue subgroup B: cracks, enamel rod end and fish scale appearance. (f)- Covarine blue subgroup B: scratches and fish scales prisms appearance. S, Scratches; E P, Enamel pores; Ca P, Calcium particles; E R, Enamel rod end; P, Perikymata; F, Fish scale appearance; C, crack (SEM, Original magnification a, c &e x1000, b, d & f x5000).

then followed by covarine blue subgroup with no significant difference between control and covarine blue subgroups and finally the lowest value was in calcium carbonate subgroup. There was no statistically significant difference between all subgroups according to weight% of Ca in 3 weeks (table 1a, figure 3a).

Subgroup B:

There was no statistically significant difference between subgroups at 7 weeks according to weight% for C and Ca. While, there was a statistical difference in mean of P, the highest weight % was in calcium carbonate subgroup followed by control subgroup and the least mean was in covarine blue subgroup (table 1b, figure 3b).

Comparison between different groups at different duration:

There was statistically significant difference of ions percentage from 3 to 7 weeks in different groups. The highest weight percentage change of carbon was recorded in control groups followed by covarine blue with no significant difference between covarine and control groups. The least value recorded in calcium carbonate groups. Regarding weight% of P, it was highest in calcium carbonate group followed by control group and the least weight recorded in covarine blue group and there was no statistically significant difference in control and covarine blue group. While the highest weight% of Ca was recorded in calcium carbonate group followed by covarine blue group, with no statically significant difference between both groups. The least weight recorded in control group with statistically difference between it and other groups (table 1c, figure 3c).

Microhardness results:

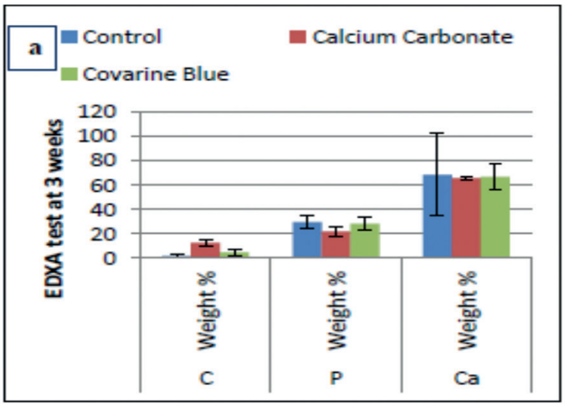
Subgroup A:

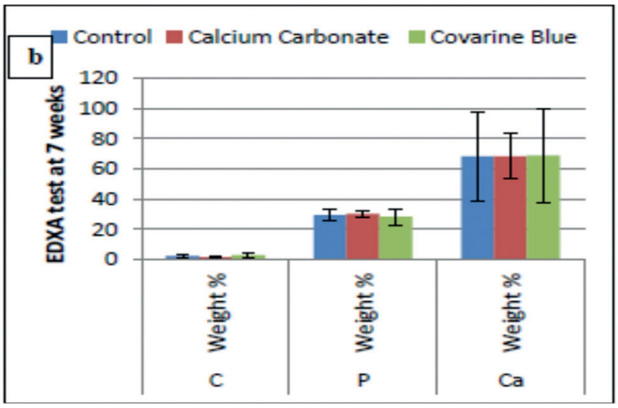
There was highly significant difference between subgroups. The highest mean of microhardness was recorded in control subgroup, followed by covarine blue, with no statically significant difference between both subgroups. The least value recorded in calcium carbonate subgroup (table 2a, figure 4a).

TABLE (1) Showing the mean ± SD values, results of ANOVA and post hoc tests for the comparison between different subgroups regarding EDXA test (a) at 3 weeks. (b) at 7 weeks. (c) amount difference between 3 and 7 weeks".

EDXA test		Control (n=5)	Calcium Carbonate (n=5)	Covarine Blue (n=5)	F-test	p-value
(a) 3 weeks	C Weight %	1.82±1.82B	12.56±2.28A	4.63±2.28B	54.364	<0.001**
	P Weight %	29.63±0.94A	21.91±2.13B	28.54±0.96A	65.732	<0.001**
	Ca Weight %	68.54±1.05	65.53±4.32	66.83±2.19	2.226	0.133
(b) 7 weeks	C Weight %	2.32±1.31	1.39±0.71	2.85±1.99	2.101	0.147
	P Weight %	29.49±0.86B	30.20±0.21A	28.36±1.05C	10.934	<0.001**
	Ca Weight %	68.19±0.58	68.41±0.85	68.80±2.47	0.314	0.734
(C) Comparison between 3&7 weeks	C Weight %	0.50±0.24A	-11.17±0.57B	-1.78±0.55A	166.017	<0.001**
	P Weight %	-0.14±0.07B	8.29±0.71A	-0.18±0.07B	139.465	<0.001**
	Ca Weight %	-0.34±0.18B	2.88±1.24A	1.96±0.59A	4.276	0.028*

Different capital letters indicate significant difference at (p<0.05) among means in the same row p-value >0.05 NS; *p-value <0.05 S; **p-value <0.001 HS





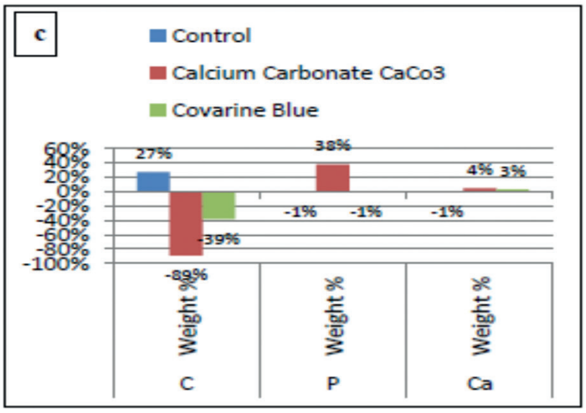


Fig. (3): Statistical chart showing: Comparison between different subgroups regarding EDXA test (a)- at 3 weeks. (b)- at 7 weeks. (c)- amount difference between 3 and 7 weeks.

TABLE (2) Showing the mean ± SD values, results of ANOVA and post hoc tests for the comparison between different subgroups regarding microhardness test (a) at 3 weeks. (b) at 7 weeks. (c) amount difference between 3 and 7 weeks.

Microhardness test		**Control (n=3)**	**Calcium Carbonate (n=3)**	**Covarine Blue (n=3)**	**F-test**	**p-value**
(a) 3 weeks	Mean±SD	356.01±8.38A	308.04±4.74B	348.71±24.93A	22.453	<0.001**
(b) 7 weeks	Mean±SD	321.46±5.36	319.68±3.87	321.18±29.58	0.024	0.976
(C) Comparison between 3&7 weeks	MD±SE	-34.55±3.41B	11.64±2.36A	-27.54±2.86B	73.133	<0.001**
	Percentage%	-9.70%B	3.80%A	-7.90%B		

MD: Mean Difference and SE: Standard error, different capital letters indicate significant difference at (p<0.05) among means in the same row, p-value >0.05 NS; *p-value <0.05 S; **p-value <0.001 HS

Subgroup B:

There was no statistical difference in microhardness mean between all subgroups (table 2b, figure 4b).

Comparison between different groups at different duration:

There was statistically significant difference between groups with the highest positive amount of change between 3 and 7 weeks was in calcium carbonate group, followed by covarine blue group. The lowest value was recorded in control group with no significant difference between it and covarine blue group (table 2c, figure 4c).

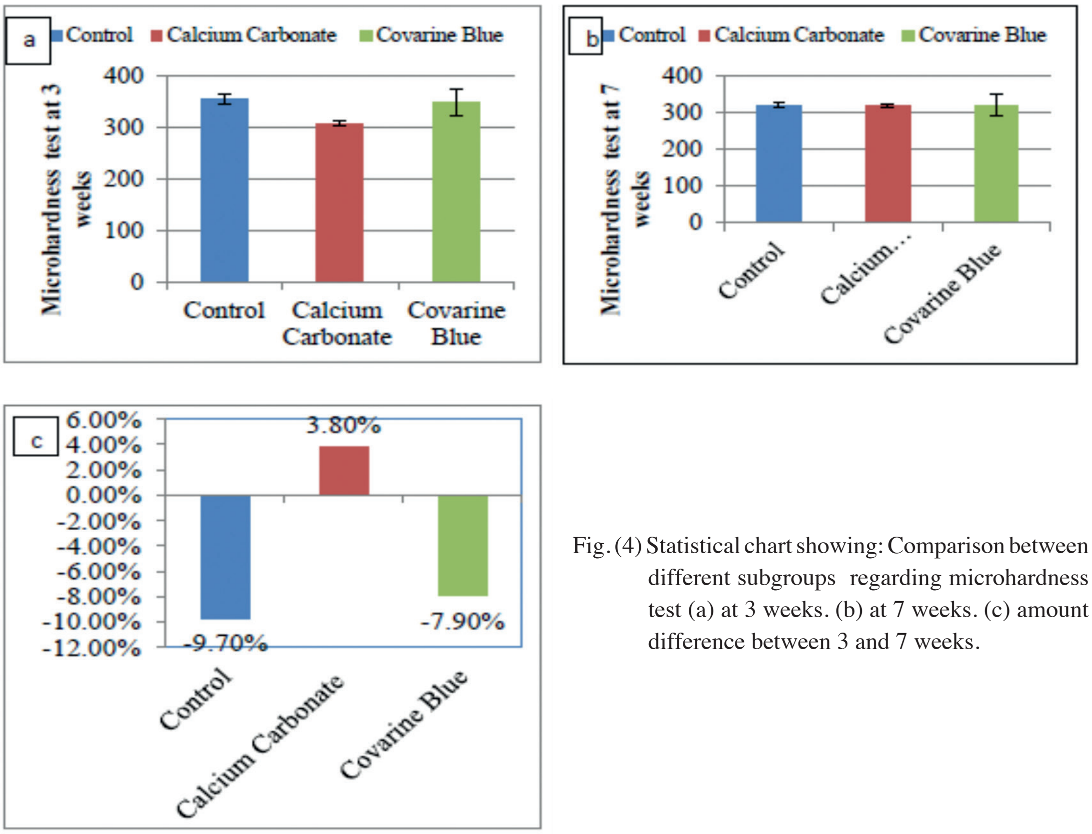


Fig. (4) Statistical chart showing: Comparison between different subgroups regarding microhardness test (a) at 3 weeks. (b) at 7 weeks. (c) amount difference between 3 and 7 weeks.

DISCUSSION

Enamel is an effective barrier that shields the pulp and dentin of teeth from damage. It guards against acid and plaque-induced tooth decay and shields the inner, sensitive tooth layers from high temperatures [13]. The use of toothpaste with whitening agents is a widely accessible method for teeth whitening. Among other over-the-counter medicines, this technique offers a commercial and alternate method of cleaning the enamel surface of the teeth of stains [14].

One of the most whitening abrasives used in toothpastes is calcium carbonate, it was used as abrasive as a combination with perlite material. Then calcium carbonate was used as the only abrasive material in toothpaste to reduce the usage of aggressive abrasive perlite [15]. The other type of whitening toothpaste which discussed in our study is the optical type using covarine blue. This type depends on film of covarine with blue color attached to tooth surface [16].

Sound human premolars with intact enamel were used in the study to provide realistic and accurate results of possible effects of the used bleaching materials. Additionally, the middle third of buccal surface of the enamel is the most affected, thus that's where the investigation was focused [10;17].

In this study, the teeth of groups were immersed in distilled water instead of artificial saliva to eliminate the remineralization effect of it, which may alter the reported results [18;19].

In the present study, electrical brushing was applied with examined material on the enamel surface and this was in accordance with **Greuling et al.,** [20] who made their study on charcoal toothpaste and decided to use electrical toothbrush to make a definite brushing power and the results showed little cracks or just scratches.

In our investigation, the best experimental method for determining the concentrations of the components under investigation in specific enamel regions was by using EDXA analysis. It links the distribution of different elements with the tissue's histological structure by non-destructively analyzing the weight percentage of an element on a few square microns [21].

Microhardness of enamel surface was assessed to know the change in enamel surface topography. The topography of the enamel surface and the light reflection pattern may be altered by the indentations, which could have an adverse impact on the analysis of color and roughness [22]. In our study as a result, the surface microhardness was only assessed at the final stage, and a statistical comparison between the experimental and control groups was made.

In the current study, SEM examination of enamel surface in calcium carbonate groups showed calcium particles precipitation that increased by time. Also, the results showed increasing of microhardness in calcium carbonate group in comparison to other groups, these findings proved the efficacy of calcium carbonate bleaching toothpastes in relief pain by increasing enamel thickness or by closing dentinal tubules in exposed areas due to calcium precipitation [23]. The increase in microhardness after 7 weeks in this group could be related to effect of calcium carbonate by increasing enamel mineralization [22].

The EDXA result in this study represented changes in ions level in calcium carbonate group and it was more detectable after 3 weeks than 7 weeks. This could be related to rapid release of calcium carbonate bleaching toothpaste particles when it exchanges with hydrogen cations (H_2O_2). The amount of sodium ions raised and the pH of the surrounding area raised when toothpaste particles were adhered to the surface of teeth. The formation of nucleation sites and the collapse of hydroxyapatite during the early stages of caries were both significantly influenced by the rise in the quantity of different ions on the enamel surface [24].

Regarding our results of enamel in covarine blue groups, it had been found that enamel surface showed similar topography as in control group. That finding is in accordance with **Greuling et al.,** (16) who used optical bleaching toothpastes and reported no obvious changes to original tooth in chemical or physical composition.

In our study, regarding ion content of C, Ca and P in covarine blue groups, there is no significant difference from 3 weeks to 7 weeks just the normal change which was very near to change in control group, this finding is in agreement with **Vyavhare et al.,** (25) who reported a little effect of bleaching toothpastes and they related these little changes on enamel to time factors, brushing activity or other components of toothpastes.

Statistically, the microhardness of covarine blue group after 3 weeks showed no significant difference to control group at 3 weeks and no significant difference to other groups at 7 weeks. These findings are in accordance with **Vieira-Junior et al.,** (22). **Joiner et al.,** (16); **Van Loveren and Duckworth,** (26) reported that optical bleaching toothpastes like covarine blue toothpastes had the minimum abrasive materials and has minimal change in microhardness as it just made thin covarine film on enamel to bleach it.

In this study, regarding to C weight, it was significantly higher in calcium carbonate group than covarine blue group after three weeks but there was no significant difference after seven weeks. This could be related to exchange of ions between C and hydroxyapatite crystals in deep enamel by using calcium carbonate containing toothpaste (24).

On the other hand, the P presented highest significant difference in calcium carbonate group in relation to covarine blue group from 3 to 7 weeks. P showed the highest increase in weight in calcium carbonate group and this was in parallel with **Altan et al.,** (24) who linked this to phosphorous ions release during brushing by calcium carbonate tooth pastes.

In this study, the microhardness of enamel surface, in covarine blue group was the highest at 3 weeks with significance difference to 7 weeks and vice versa in calcium carbonate group. There was no significant difference between the two groups after seven weeks brushing. This change may be associated to precipitation of calcium ions on enamel surface and increasing media alkalinity as researched by **Lynch and Tencate** (5); **Attin et al.,** (27).

CONCLUSIONS

By SEM examination, calcium carbonate group showed precipitation of calcium on enamel surface but there was no obvious difference between covarine blue and control group. EDXA analysis after 3 weeks from toothpastes application represented increase in P and C in covarine blue and calcium carbonate respectively. While after 7 weeks, P ions increased only after applying of calcium carbonate toothpaste. Application of calcium carbonate tooth paste increase the difference of microhardness of enamel surface from 3 to 7 weeks in comparison to covarine blue.

RECOMMENDATIONS

Further researches comparing the two toothpastes with different concentrations, and for longer duration on ions weight changes and on the structure of different hard dental tissue are suggested.

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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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