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Comparing the Effect of Activated Charcoal Whitening Agents against Regular Whitening Toothpastes on the Enamel Surface of Premolar Teeth: *In vitro* Study

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Abstract

BACKGROUND: There has been increase in demand to use natural whitening agents nowadays which include activated charcoal. Activated charcoal has gained popularity recently with various claims to its benefits that have not been proven.

AIM: The aim of the study was to evaluate the effect of activated charcoal whitening agents and regular whitening tooth paste on enamel surface of first premolars and to compare between the effects of materials used.

METHODS: Twenty-one mandibular first premolars were used and divided into control group: untreated teeth that were later incorporated into experimental group. Experimental group: Consisting of three subgroups: Subgroup I: treated with Carbon Coco. Subgroup II: treated with Venu activated charcoal. Subgroup III: treated with Crest 3D white. Brushing was done nine s for 30 days then teeth were preserved in artificial saliva.

RESULTS: Scanning electron microscopic results showed areas with rodless enamel more observed in Subgroup III than Subgroups I and II. EREs were more numerous on Subgroups I and II than Subgroup III on enamel surface. Partially occluded EREs with precipitates were more observed on Subgroups I and II than Subgroup III. Energy dispersive X-ray analysis results for calcium showed significant increase between all groups with control. The highest value was recorded in Subgroup II followed by Subgroups I and III while control group was the lowest one. Phosphorous showed significant increase between all groups with control. The highest value was recorded in Subgroup I followed by Subgroups III and II while control group was the lowest one. Micro-hardness results of Subgroups I and II showed significant increase compared to control group and no significant difference between control and Subgroup III was detected.

CONCLUSION: Powder form activated charcoal is more abrasive than whitening toothpastes. There is no visible difference between pure 100% activated charcoal powder and charcoal powder with additives to it.

Introduction

Tooth discoloration could occur in the form of intrinsic stain inside the tooth structure or as an extrinsic stain that may form on the enamel surface [1].

Whitening can treat discoloration with an assortment of techniques: In-office bleaching, home-bleaching, and over the counter bleaching products. Over the counter products are a low cost alternative to other bleaching techniques and are available at pharmacies, supermarkets, and over the internet [1]. Despite their many advantages, some of their ingredients have a harmful side to them [2].

As a result, general population began leaning more toward natural products as they are free of chemicals; hence, free of harmful side effects [3], [4].

In addition, some people choose to whiten their teeth using homemade remedies and traditional methods that are cheap and easily attainable. These methods have swept through the internet gaining popularity [4]. One of which is activated charcoal which has been used for whitening of tooth surface as well as its use in many beauty hacks [5].

Different manufacturers have claimed that charcoal has benefits in cosmetic and health fields. These claims include its possible use as antibacterial, antifungal, or antiviral. For caries reduction as it is claimed to have the ability to absorb plaque and to clean the teeth including interdental areas, which are difficult to reach. In addition, they also claimed that it was safe for oral use. However, those claims have not been scientifically proven which poses a considerable concern of these products credibility [6], [7].

Since, activated charcoal is claimed to have a similar mode of action, in terms of abrasiveness, to regular whitening tooth paste. Thus, this study evaluated the effect of two different activated charcoal whitening agents and regular whitening tooth paste on enamel surface of mandibular first premolars.

Materials and Methods

This experimental design was revised by the Research Ethics Committee of the faculty of dentistry Ain-Shams University and received exemption its number is: FDASU-RecED021910.

Materials

Twenty-one recently extracted mandibular first premolars were selected and stored after cleaning and removing debris in distilled water until testing commenced.

The teeth were randomly divided into two groups:

Control Group: Consisting of seven sound untreated mandibular first premolars which were then incorporated into the experimental group.

Experimental Group: Divided into three sub-groups (seven premolars each): Subgroup I: Was treated with Carbon Coco. Subgroup II: Was treated with Venu-activated charcoal. Subgroup III: Was treated with Crest 3D white.

Preparation

In the middle third of the buccal surfaces of all teeth, a window 2 × 3 mm was created by covering selected area with pink wax then the remaining of buccal surfaces was covered by nail varnish. After the varnish application, the pink wax was removed. The paste of Carbon Coco and Venu was prepared by mixing equal amounts of powder with water until a proper paste is obtained according to manufacturer's instructions. Oral B electronic toothbrush with precision clean head was dipped into the paste mixture or in toothpaste in Subgroup III and the middle third of buccal surface of teeth of experimental subgroups was brushed for 9 s. Then, buccal surface was cleaned using distilled water. This procedure was repeated once daily for 30 consecutive days [7]. While preserving teeth in artificial saliva between washes, artificial saliva was prepared at faculty of pharmacy Ain Shams University according to the formulation of Vieira *et al*., (2016) [8]. Teeth were refrigerated until examined.

Examination

Scanning electron microscopy (SEM) was performed at desert research center in El Matareya by SEM machine model Quanta 250 FEG (field emission gun) attached with energy dispersive X-ray analysis unit (EDXA). Evaluation of enamel surface was done at magnifications 1000 and 4000. Energy dispersive X-ray analysis was performed to evaluate the levels of calcium (Ca) and phosphate (P) minerals. Micro-hardness testing was performed at October University for Modern Sciences and Arts laboratories with load of 200g for 15s [6] on enamel surface.

Statistical analysis for EDXA results and micro-hardness test

Sample size calculation was performed using G*Power version 3.1.9.2, Faul *et al*., (2007) [9]. The effect size d was 0.95 using alpha (α) level of 0.05 and beta (β) level of 0.05, i.e., power = 95%; the estimated sample size (n) should be 21 samples for this study.

All data were collected, calculated, tabulated, and statistically analyzed using the following statistical tests. A normality test (Shapiro–Wilk) was done to check normal distribution of samples. Descriptive statistics were calculated in the form of Mean ± Standard deviation (SD). One-way ANOVA was used to compare between groups. Bonferroni *post hoc* tests were performed to evaluate the statistical significance among the factors. $p < 0.05$ is considered be statistically significant. All analysis was done using computer program SPSS software for windows version 26.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp) at significant levels 0.05 ($p \leq 0.5$).

Results

The SEM examination of the middle third of the buccal surface of the lower first premolar in control group revealed few Perikymata grooves (PG) and ridges (PR) with observed enamel rod ends (EREs). Areas of rodless enamel were observed on the Perikymata ridges and they appeared as areas with no structure and with a uniform surface. The EREs appeared as areas of shallow saucerization of the enamel surface. This saucerization almost had regular margins (Figure 1a and b).

Subgroups I and II revealed pitted enamel surface with less defined Perikymata ridges and grooves and fewer areas with rodless enamel (Figures 1c and 2a).

It also showed numerous EREs on the enamel surface, some with defined borders, others with irregular boundaries. Partially occluded EREs were also observed on enamel surface. Some precipitates were deposited on top of the EREs, others were encountered in the concavities of EREs, which presumably lead to partial occlusion of some EREs (Figure 1d and 2b).

The SEM results of Subgroup III also showed pitted enamel surface. However, EREs were apparently less numerous than Subgroups I and II with irregular boundaries. Partially occluded EREs were also observed on enamel surface with precipitates deposited on top of EREs, others were encountered in the concavities of EREs. These precipitates were apparently less

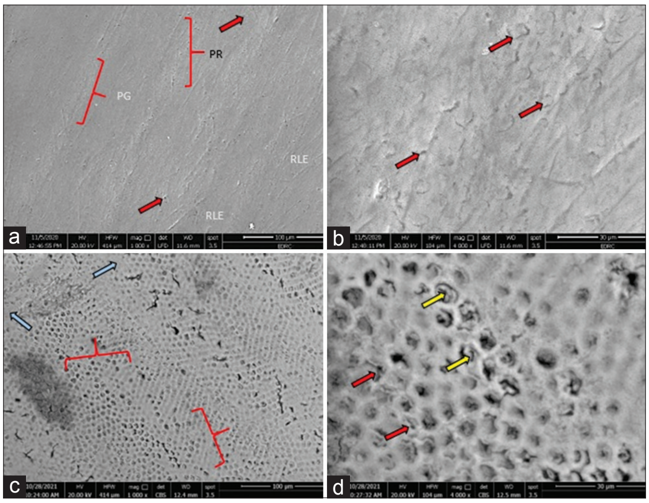

Figure 1: (a) SEM of control group showing Perikymata grooves (PG) and ridges (PR) with EREs (red arrows) and areas of rodless enamel (RLE) (×1000). (b) A higher magnification of 1A showing EREs (red arrows) (×4000). (c) SEM of experimental Subgroup I showing ill-defined Perikymata (bracket) and areas with few rodless enamel (blue arrow) (×1000). (d) A higher magnification of 1c showing EREs with irregular prism boundaries and precipitates on top them (red arrow) and others precipitated in the concavities of the EREs (yellow arrows) (×4000)

observed than Subgroups I and II. Perikymata was also less defined but, areas with rodless enamel were apparently fewer yet, more observed than Subgroups I and II (Figure 2c and d).

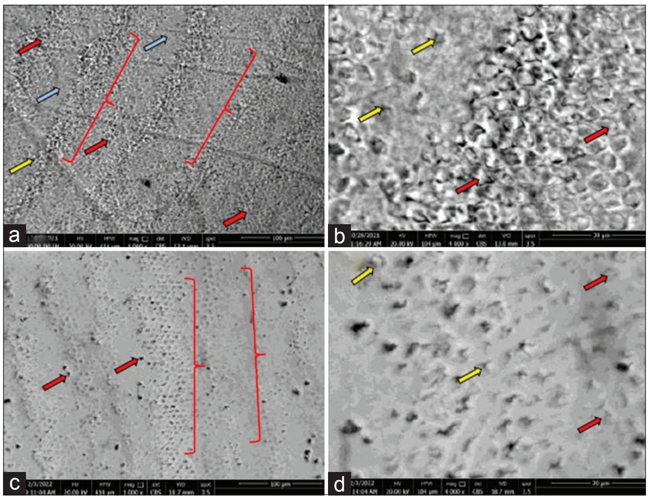

Figure 2: (a) SEM of experimental Subgroup II showing pitted enamel (yellow arrow), numerous EREs (red arrow), ill-defined Perikymata (brackets) and areas with few rodless enamel (blue arrow) (×1000). (b) A higher magnification of 2A showing irregular prism boundaries (yellow arrows) and regular prism boundaries (red arrow) (×4000). (c) SEM of experimental subgroup III showing areas of EREs (red arrow) and ill-defined Perikymata (brackets) (×1000). (d) A higher magnification of 2C showing EREs with irregular prism boundaries and precipitates on top them (red arrow) and others precipitated in the concavities of the EREs (yellow arrows) (×4000)

The EDXA results for Ca showed significant increase between all studied groups with control group. The highest value was recorded in Subgroup II followed by Subgroup I and Subgroup III while control group was the lowest one (Table 1 and Figure 3a). Moreover, P element showed significant increase between all studied groups with control group. The highest value was recorded in Subgroup I followed by Subgroup III and Subgroup II while control group was the lowest one (Table 2 and Figure 3b).

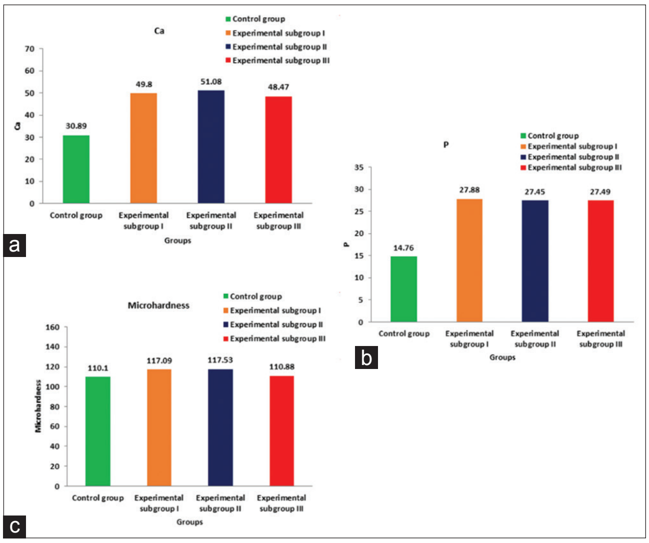

Figure 3: (a) Bar chart comparing between subgroups for Ca element. (b) Bar chart comparing between subgroups for P element. (c) Bar chart comparing between subgroups for micro-hardness

Micro-hardness results of Subgroups I and II showed significant increase when compared to control group and no significant difference between control and Subgroup III was detected (Table 3 and Figure 3c).

Discussion

In recent years, products containing activated charcoal had attracted the interest of researchers and general population due to their alleged whitening and cleansing effect [10].

Few studies were published concerning the effects of products containing activated charcoal on enamel [11]. Therefore, in this study, we evaluated the effect of activated charcoal whitening agents and regular whitening tooth paste on the enamel surface of first premolars.

In this study, SEM examination results of Subgroups I and II agree with a previous study [12] that observed prominent porosities and visible depressions throughout the enamel surface in group with powder activated charcoal.

A previous study [13] observed heterogeneous enamel surface with numerous large and deep craters and increased roughness in enamel brushed with charcoal-containing toothpaste. These results were consistent with our results despite the fact that they observed enamel surface with confocal microscope while in our study, we used SEM. Meanwhile, another

Table 1: Comparison between subgroups regarding Ca

Group	Mean ± SD	F-test	p	
Control group	30.89[b] ± 8.47	25.28	< 0.001**	
Subgroup I	49.80[a] ± 2.93			
Subgroup II	51.08[a] ± 3.61			
Subgroup III	48.47[a] ± 2.59			
Multiple comparisons using Tukey *post hoc*				
Pair wise	Mean difference	95% CI		p
		Lower bound	Upper bound	
Control group versus Subgroup I (Carbon Coco)	−18.91	23.05	38.73	< 0.001**
Control group versus Subgroup II (Venu activated charcoal)	−20.19	47.08	52.51	< 0.001**
Control group versus Subgroup III (Crest 3D white)	−17.58	47.74	54.42	< 0.001**
Subgroup I (Carbon Coco) versus Subgroup II (Venu activated charcoal)	−1.28	46.08	50.86	0.963
Subgroup I (Carbon Coco) versus Subgroup III (Crest 3D white)	1.33	23.05	38.73	0.959
Subgroup II (Venu activated charcoal) versus Subgroup III (Crest 3D white)	2.61	47.08	52.51	0.765

p < 0.05: NS: Non-significant, CI: Confidence interval, SD: Standard deviation.

study [14] reported that enamel surface was smooth after application of powder based activated charcoal. These results were different from ours, which could be explained by the duration of the experiment. In our study, the brushing period was 1 month while, in their work [14] the brushing period was 14 days.

Moreover, a previous research [15] observed almost smooth enamel surface with few scratches. These findings were dissimilar from our results. The difference could be due to their use of whitening tooth paste containing activated charcoal in their work while we used powder form.

In the present study, subgroup III results showed that abrasion occurred on the enamel surface but with a lesser degree than Subgroups I and II. These findings coincide with the findings of another study [12] who also found that regular and whitening toothpastes did not cause the same abrasive effect as activated charcoal powder.

In addition, some researchers [13] observed that brushing of the enamel with the charcoal-containing toothpaste induced different surface morphology patterns compared to the regular toothpaste which contains hydrated silica.

All our findings indicated that activated charcoal can induce surface changes to enamel which could be more pronounced than regular whitening tooth pastes. These results can be verified by the surface roughness test performed by the previous studies [7], [12], [15] who confirmed that activated charcoal cause changes to the enamel surface.

Not many investigated the effect of activated charcoal on the micro-hardness of enamel. In our study, micro-hardness results were dissimilar to the findings of a previous study [15] in which the micro-hardness results showed no alteration. This could be explained by the fact that they [15] used darkening procedure.

Another research work [6] was also different from ours in which, they reported decrease in micro-hardness for groups containing activated charcoal. This difference could be explained by the fact by [6] the exposure of enamel surface to sucrose solution containing the strain *Streptococcus mutans* in their study which, caused demineralization of enamel surface.

According to a previous study [16], micro-hardness is related to remineralizing capacity of the artificial saliva used as a storage solution. Sufficient storage time can repair any defect caused to the enamel surface which was proven by the presence of calcium-phosphate precipitation inside the enamel pores. This process can maintain the micro-hardness of the enamel surface. In our study, we used artificial saliva as a storage medium which could explain the increase in micro-hardness observed in subgroups I and II.

Moreover, other research [17] observed increase in micro-hardness results in toothpaste containing abrasive component. This increase indicted that the remineralization rate is higher than their demineralization rate caused by these abrasives. Thus, the previous studies explained the increase in micro-hardness results found in our study despite the fact that they did not use activated charcoal.

Energy dispersive X-ray analysis is usually performed to evaluate the mineral content of the tissue being evaluated which in our study is enamel. Not many studies performed EDXA on enamel treated with activated charcoal.

Table 2: Comparison between subgroups regarding P

Group	Mean ± SD	F-test	p	
Control group	14.76[b] ± 5.69	30.31	< 0.001**	
Subgroup I	27.88[a] ± 1.07			
Subgroup II	27.45[a] ± 1.61			
Subgroup III	27.49[a] ± 1.42			
Multiple comparisons using Tukey *post hoc*				
Pair-wise	Mean difference	95% CI		p
		Lower bound	Upper bound	
Control group versus Subgroup I (Carbon Coco)	−13.12	9.51	20.02	< 0.001**
Control group versus Subgroup II (Venu activated charcoal)	−12.68	26.89	28.87	< 0.001**
Control group versus Subgroup III (Crest 3D white)	−12.72	25.95	28.94	< 0.001**
Subgroup I (Carbon Coco) versus Subgroup II (Venu activated charcoal)	0.44	26.17	28.80	0.993
Subgroup I (Carbon Coco) versus Subgroup III (Crest 3D white)	0.40	9.51	20.02	0.995
Subgroup II (Venu activated charcoal) versus Subgroup III (Crest 3D white)	−0.04	26.89	28.87	0.999

p < 0.05: Non-significant. NS: Nonsignificant, CI: Confidence interval, SD: Standard deviation.

Table 3: Comparing micro hardness values between different subgroups

Group	Mean ± SD	F-test	p	
Control group	110.10[b] ± 2.36	8.48	0.0072**	
Subgroup I	117.09[a] ± 1.75			
Subgroup II	117.53[a] ± 3.03			
Subgroup III	110.88[b] ± 2.07			
Multiple comparisons using Tukey *post hoc*				
Pair-wise	Mean difference	95% CI		p
		Lower bound	Upper bound	
Control group versus Subgroup I (Carbon Coco)	−6.987	104.23	115.97	0.027**
Control group versus Subgroup II (Venu activated charcoal)	−7.427	112.75	121.42	0.020**
Control group versus Subgroup III (Crest 3D white)	−0.783	110.00	125.05	0.976
Subgroup I (Carbon Coco) versus Subgroup II (Venu activated charcoal)	−0.440	105.74	116.02	0.995
Subgroup I (Carbon Coco) versus Subgroup III (Crest 3D white)	6.203	111.30	116.50	0.045**
Subgroup II (Venu activated charcoal) versus Subgroup III (Crest 3D white)	6.643	104.23	115.97	0.035**

$p < 0.05$: NS: Non-significant, CI: Confidence interval, SD: Standard deviation.

A study [18] found that there was a direct connection between micro-hardness of the tooth and it's mineral content. Loss in Ca and P levels causes change in micro-hardness of enamel. Their study was done on enamel surface of different age groups. This coincides with our EDXA results despite the fact that the previous study's [18] work was dissimilar to ours. In our study, the experimental groups showed higher values in both micro-hardness and EDXA than control group which showed the lowest value.

Another study [19] observed a correlation between the SEM, EDXA analysis, and micro-hardness results. Increased micro-hardness corresponded to increase in mineral content which, in turn, results in SEM image with deposition of minerals and decrease in pore volume. In their study, they used different mineralizing tooth pastes on demineralized enamel.

Although the previous study's [19], design was dissimilar to ours, the correlation that they observed could explain our results. In our study, the groups that showed increase in Ca and P also showed increase in micro-hardness and deposition of precipitates in SEM.

In our study, the increase in Ca and P levels seen in EDXA analysis of Subgroups I and II could be explained by the restorative capacity of artificial saliva which has the capacity to restore defects caused by abrasive action of activated charcoal. It was further explained that the remineralizing ability of the saliva is due to presence of saturated amounts of Ca and P elements in its composition [20].

Our EDXA results showed differences between both activated charcoals (Subgroups I and II) this could be explained by the difference in composition between Subgroups I and II in which, Subgroup I has additional components, which could explain the difference in results.

Conclusions

Activated charcoal is a strong abrasive with no visible difference between pure powder form and powder with additives. Powder activated charcoal is more abrasive than whitening toothpaste. Saliva can remineralize the enamel surface and counteract the damage done.

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