Original Article

Remineralization Activities of Toothpastes with and without Aloe Vera with Different Ratios of Fluoride on Demineralized Enamel: An *In-vitro* Study

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Abstract

AIM and Background: The aim of this *in-vitro* study was to evaluate and compare the efficacy of both pure aloe vera and commercially available toothpastes with different fluoride compounds and different fluoride amounts on artificial initial enamel lesions by Vicker's microhardness values. In the study, 72 extracted human molar teeth were divided into mesiodistal and 144 specimens were prepared using the vestibule and palatal/lingual surfaces of the teeth. After the surface treatments and initial microhardness measurements, all the specimens were placed in a demineralizing solution (pH: 4.5) for 7 days, resulting in artificial initial enamel lesion, and were randomly assigned to eight groups (n = 18). After the teeth were subjected to pH cycle for 14 days, microhardness measurements were repeated and the data were recorded. **Materials and Methods:** Statistical analyzes were performed using MedCalc Statistical Software version 12.7.7. The significance level was determined to be 0.05. **Results:** In the statistical results, when the microhardness values after demineralization and post-cycle were compared, Groups B1 and A2 showed the lowest values, while Groups A3 and B3 did not show a significant difference in terms of microhardness values after demineralization and post-cycle, and only Group B4 showed statistically significantly higher values. **Conclusions:** This study emphasized the remineralization effects of fluoride on initial enamel lesions. It can be said that toothpaste containing 1450 ppm fluoride and aloe vera provides an effective remineralization and sodium monofluorophosphate formulation may have a synergistic effect with aloe vera.

Keywords: *Aloe vera, fluoride, microhardness, prevention, remineralization, toothpaste*

Introduction

White spot lesions are early signs of demineralization under intact enamel. It is very important to remineralize these lesions; otherwise, this can lead to the development of caries.[1] Minimally invasive dentistry is a concept that emerged as a result of a better understanding of the caries process and advances in adhesive dentistry. This concept basically includes remineralization of initial caries lesions, reduction of cariogenic bacteria in order to eliminate the risk of further demineralization and cavitation, minimalize of surgical intervention of cavitated lesions, and repair rather than replacement of defective restorations and disease control.[2]

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Use of fluorides has been the most important caries prevention method since the introduction of water fluoridation in the 1940s.[3] At the present time, traditional fluoride-based remineralization remains the cornerstone for caries management but also fluoride has disadvantages. Novel remineralizing technologies are categorized as biomimetic regenerative systems, approaches that synergize fluoride activity and natural products.[4]

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Aloe vera is widely used in dentistry as a natural consequence of its wide range of effects such as oral mucosal diseases,[5] pulpatomy,[6] alveolar osteitis,[7] against *Enterococcus faecalis,*[8,9] potential root canal filling material in primary teeth,[10] in many field of periodontology,[11-13] disinfection of gutta percha cones, denture adhesive formulations, and disinfection of irrigation units.[14]

With the increasing interest in natural products, research in the field of remineralization has increased. Considering the limited studies on the remineralization potential of aloe vera, the aim of this study is to evaluate the demineralization-remineralization efficiencies of toothpastes with different fluoride formulations and different fluorine amounts on demineralized enamel under *in-vitro* conditions by Vicker's microhardness values.

Subjects and Methods

Ethical approval

This *in-vitro* study was conducted between 2020 and 2021 in Ankara, Turkey. Ethical approval was obtained from the Gazi University Faculty of Dentistry Clinical Research Ethics Committee on 16.07.2020 with the decision number 2020.15/3.

Power analysis

Sample-size calculations were made in the package program (G*Power 3.1.9.6., Franz Faul, Universitat Kiel, Germany). In order to test statistical significance at 40% effect size, 80% power, 5% tolerance, and 1/4 possible data loss were taken into account and 18 samples were calculated to be included in each group.

Selection and preparation of the sample

Attachments on the extracted permanent human third molar teeth were removed mechanically and cleansed using pumice and a polishing brush. The 72 human third molar teeth were inspected for caries, fracture, crack, restoration, demineralization, hypomineralization, and wear.

The teeth were separated from the cemento-enamel junction and mesiodistally under water cooling, allowing the use of both buccal and palatinal surfaces. The specimens were embedded with their buccal or palatinal surfaces upward in an acrylic resin.

The enamel surfaces were polished in a polishing device (Mecapol P230, Presi, France) using a sequence of 400, 600, and 1200 grit silicon carbide paper abrasives (Atlas Abrasives, İstanbul, Turkey), then coated in two layers with a nail polish, leaving a narrow 3 mm × 3 mm wide window on the polished surface of the enamel.[15]

Measuring surface microhardness

The surface hardness of the enamel was determined using a Vicker's microhardness tester (Shimadzu HMV-700 Shimadzu, Kyoto, Japan) under a 200-g load for 10 s.[16,17]

Results were obtained from three different regions of each sample and mean values were recorded. Microhardness values were measured and recorded at the beginning, after demineralization, and post-cycle.

Demineralization of the sample

All samples were incubated in demineralization solution at 37°C for 7 days. At the end of 7 days, the samples were gently rinsed under running water without damaging the surface, and after drying the samples, artificial enamel lesions were observed on the surfaces.

The demineralizing solution contained 2.2 mM calcium chloride ($CaCl_2$), 2.2 mM sodium dihydrogen phosphate (NaH_2PO_4), 50 mM acetic acid (CH_3COOH), and had a pH adjusted to 4.5 at 37°C with 1 M potassium hydroxide (KOH) and prepared fresh daily.[18]

Test groups and active ingredients

The samples were randomly divided into two main groups and then to four subgroups as follows, with similar initial and after demineralization microhardness values (n = 18/group): Group A1, no treatment (distilled water); Group A2, fluoride-free toothpaste (R.O.C.S. Junior; WDS, Moscow, Russia); Group A3, toothpaste with 1100 ppm fluoride (Oral-B KIDS, P&G, Gross-Gerau, Germany); Group A4, toothpaste with 1450 ppm fluoride (Colgate Total, Colgate-Palmolive, Guangzhou, China); Group B1, aloe vera leaf gel; Group B2, fluoride-free toothpaste with aloe vera (SPLAT Special Organic Toothpaste, Novgorod, Russia); Group B3, 1000 ppm fluoride toothpaste with aloe vera (LR Aloe vera KIDS, LR Health & Beauty, Ahlen, Germany); and Group B4, 1440 ppm fluoride toothpaste with aloe vera (LR Aloe vera sensitive protect toothpaste, LR Health & Beauty, Ahlen, Germany).

The active ingredients of the test groups are as follows: Group A2, xylitol (12%), calcium glycerophosphate, *Aloe barbadensis* leaf extract; Group A3, trisodium phosphate, sodium phosphate, sodium fluoride (1100 ppm), limonene; Group A4, arginine, tetrasodium pyrophosphate, sodium fluoride (1450 ppm); Group B2, calcium lactate, limonene; Group B3, *A. barbadensis* extract, xylitol, calcium glycerophosphate, sodium fluoride (1000 ppm); and Group B4, *A. barbadensis* leaf juice, calcium carbonate, sodium monofluorophosphate (1440 ppm).

Preparation of experiment materials

In the study, the test materials were applied by obtaining a slurry with distilled water at the rate of one-third (toothpaste: distilled water) by weight.[19] Toothpaste and distilled water, the weight of which was determined with a precision scale (Precisa XB 220A; Precisa Instruments, Dietikon, Switzerland), were blended in the tube. The suspensions were thoroughly stirred and mechanically shaken by mixing device (Advanced Vortex ZX3, Velp Scientifica Sri, Usmate, Italy).

The fresh aloe vera leaves were collected from the plant and washed in the running tap water. Then they were dissected longitudinally and the colorless parenchymatous tissue, that is, aloe-gel, was scraped out using sterile knife and gel-like pulp was separated with spoon, crushed, and homogenized.[20,21] All experimental materials were freshly prepared and applied.

pH cycle

The cyclic treatment regimen for each day is shown in Table 1.[22]

It consists of two 2-min toothpaste treatment periods (approximately 1 h to complete all groups for each treatment periods), one 6-h acid challenge, and then storage in remineralizing solution for the rest of the time (16-h), including night. Experimental materials were applied for 2 min by creating vibration on the vibration device (Mikrotek dental, RC-404, Ankara, Turkey) in order to imitate the flow occurring in the mouth while brushing the teeth. Specimens were treated with freshly prepared slurries of toothpaste two times per day. This daily pH cycling regimen was repeated for a total of 14 days with the human enamel.

The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 50 mM CH_3COOH and had a pH adjusted to 4.5 at 37°C with 1 M KOH and the remineralization solution contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 150 mM potassium chloride (KCl) and had a pH adjusted to 7.0 at 37°C with 1 M KOH.[18,23] Before each measurement, the pH meter (ADWA AD12, Szeged, Hungary) was calibrated using pH: 4 and pH: 7 buffer solutions (ChemBio Laboratory research, Istanbul, Turkey).

Statistical analysis

Descriptive statistics were used to describe continuous variables (mean, standard deviation, minimum, median, and maximum). The Wilcoxon signed-rank test was used for the relationship between dependent and non-normally distributed continuous variables. The comparison of more than two independent and non-normally distributed variables was made with the Kruskal–Wallis test. The comparison of two independent and non-normally distributed variables was made with the Mann–Whitney *U* test. Statistical significance level was determined as 0.05. Analyzes were performed using MedCalc Statistical Software version 12.7.7 (MedCalc Software Bvba, Ostend, Belgium; http://www.medcalc.org; 2013).

Results

In this study, the remineralization activities of different toothpastes with/without aloe vera and pure aloe vera were evaluated on extracted human tooth enamel samples using Vicker's hardness measurement method.

Vicker's hardness values of control (Group A1–distilled water), pure aloe vera gel (Group B1), without fluoride (Group A2), with aloe vera without fluoride (Group B2), 1100 ppm fluoride (Group A3), 1000 ppm fluoride and aloe vera (Group B3), 1450 ppm fluoride (Group A4), and 1440 ppm fluoride and aloe vera (Group B4) containing groups were evaluated in study.

The mean, standard deviation, minimum, maximum, and median values of the surface microhardness (SMH) values of eight groups at the beginning, after demineralization, and post-cycle, together with the results of intragroup [beginning vs. after demineralization, beginning vs. post-cycle, after demineralization vs. post-cycle (Wilcoxon test)] and intergroup (Kruskal–Wallis test) comparisons, are summarized in Table 2.

In intergroup comparisons (Kruskal–Wallis test: *P*), there was no statistically significant difference in the mean microhardness values obtained from all groups at the beginning and after demineralization ($P > 0.05$), while significant differences were observed in the post-cycle values ($P < 0.001$). The lowest post-cycle mean SMH value was observed in the pure aloe vera

Table 1: pH cycling treatment sequence for the experiment

Time	Treatment
2 min (starts 8:00 am) Approximately 1 h to complete all groups	Treatment with experiment material
1 min	Rinsed under running water
6 h (9:00 am-3:00 pm)	Acid challenge (demineralization)
1 min	Rinsed under running water
2 min (starts 3:00 pm) Approximately 1 h to complete all groups	Treatment with experiment material
1 min	Rinsed under running water
16 h (from 4:00 pm till 8:00 am next day)	Storage in remineralization solution

Repeat for 13 additional days

Table 2: The mean, standard deviation, minimum, maximum, and median values of the surface microhardness values of eight groups at the beginning, after demineralization, and post-cycle, together with the results of intragroup (Wilcoxon test) and intergroup (Kruskal–Wallis test) comparisons

	Mean±SD Med (min–max)			P^1	P^2	P^3
	Beginning	After demineralization	Post-cycle			
A1	350.89±29.05 346.17 (297.33-409)	201±55.36 192.33 (114-323.33)	43.11±25.15 35.05 (13.1-121)	**<0.001**	**<0.001**	**<0.001**
A2	343.44±29.05 343 (270-383.67)	202.61±58.54 190.83 (116-299)	23.09±7.19 23.37 (12.3-37.1)	**<0.001**	**<0.001**	**<0.001**
A3	345.74±29.84 339.5 (292.67-404.67)	192.54±46.29 176.67 (136.67-287.33)	181.86±42.27 178.5 (100.57-262)	**<0.001**	**<0.001**	0.728
A4	350.06±32.27 347.17 (272.33-414.33)	204.76±47.88 200.67 (140.67-294)	152.39±61.51 132.17 (97.3-358.33)	**<0.001**	**<0.001**	**0.003**
B1	345.94±26.44 346 (297.33-393)	197.7±66.6 182.67 (121.67-310.67)	20.53±6.54 18.68 (9.8-29.27)	**<0.001**	**<0.001**	**<0.001**
B2	343.3±24.21 344.5 (297.33-401.67)	196.95±46.48 202.67 (123-286)	36.36±12.63 37.27 (14.83-54.27)	**<0.001**	**<0.001**	**<0.001**
B3	347.31±33.26 350 (248.33-390.67)	194.59±48.72 188.67 (126.67-282.33)	189.82±41.26 185.67 (100.13-243)	**<0.001**	**<0.001**	0.556
B4	346.11±27.33 340.5 (296-387.67)	194.09±56.54 177.83 (121.67-319.67)	261.91±63.88 283.5 (130.67-337)	**<0.001**	**<0.001**	**0.001**
P4	0.982	0.993	**<0.001**			

Wilcoxon test (intragroup comparisons: [1]beginning versus after demineralization, [2]beginning versus post-cycle, [3]after demineralization versus post-cycle), Kruskal-Wallis test ([4]intergroup comparisons)

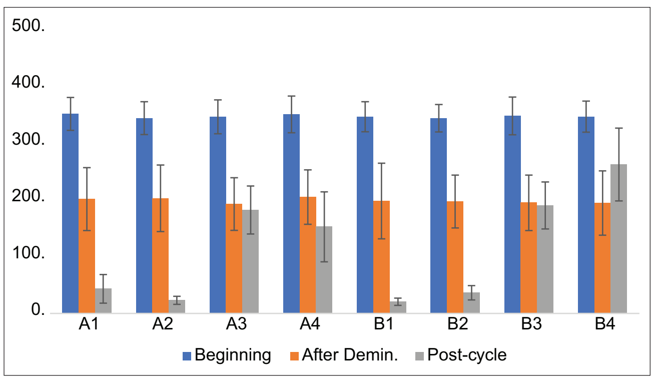

Figure 1: The mean surface microhardness values and standard deviation of all groups

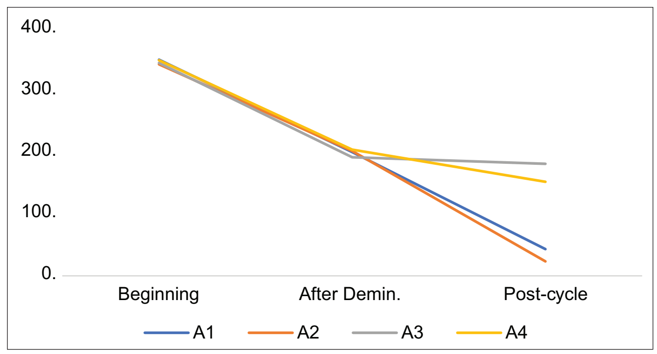

Figure 2: The mean microhardness values of groups without aloe vera (Group A)

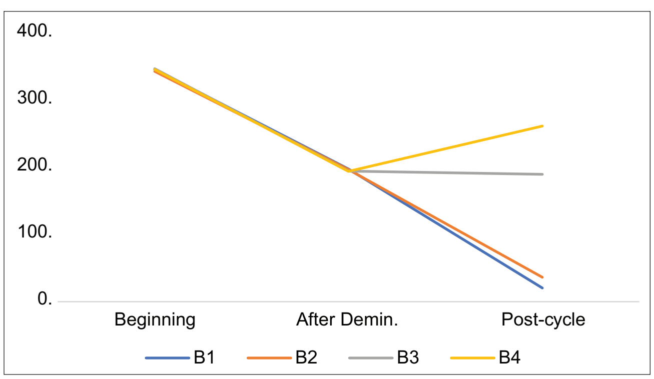

Figure 3: The mean microhardness values of groups with aloe vera (Group B)

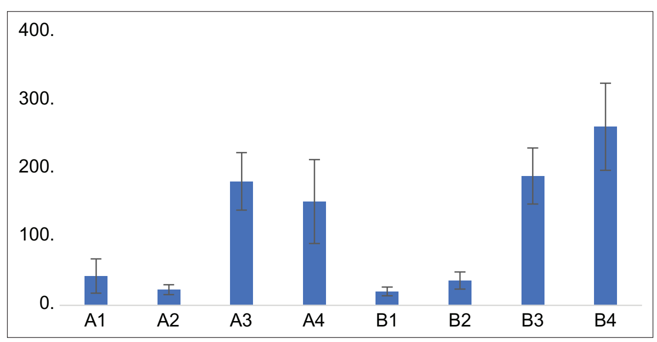

Figure 4: The mean surface microhardness values and standard deviation of all groups post-cycle

gel group (Group B1: 20.53), and the highest SMH value was observed in the toothpaste group containing 1440 ppm fluoride and aloe vera (Group B4: 261.91).

The mean and standard deviation values of all groups are shown in Figure 1, mean microhardness values of groups without aloe vera (Group A) are shown in Figure 2, mean microhardness values of groups with aloe vera (Group B)

Table 3: *Post-hoc* pairwise comparison of mean post-cycle microhardness values

Post-hoc pairwise comparison	Post-cycle
A1 versus A2	**<0.001**
A1 versus A3	
A1 versus A4	
A1 versus B1	
A1 versus B2	0.606
A1 versus B3	**<0.001**
A1 versus B4	
A2 versus A3	
A2 versus A4	
A2 versus B1	0.389
A2 versus B2	**0.001**
A2 versus B3	**<0.001**
A2 versus B4	
A3 versus A4	0.012
A3 versus B1	**<0.001**
A3 versus B2	
A3 versus B3	0.481
A3 versus B4	**<0.001**
A4 versus B1	
A4 versus B2	
A4 versus B3	0.002
A4 versus B4	**<0.001**
B1 versus B2	
B1 versus B3	
B1 versus B4	
B2 versus B3	
B2 versus B4	
B3 versus B4	**0.001**

are shown in Figure 3, mean surface microhardness values and standard deviation of all groups post-cycle are shown in Figure 4, and standard deviation and median values of all groups summarized in Figure 5. Statistically significant difference ($P < 0.05$) was observed in all intragroup comparisons (Wilcoxon test) except Group A3, after demineralization versus post-cycle (P: 0.728), and Group B3, after demineralization versus post-cycle comparisons (P: 0.556).

Statistically significant differences were observed between all groups ($P \leq 0.001$) in *post-hoc* pairwise comparison of post-cycle mean microhardness values except for A1 versus B2 ($P = 0.606$), A2 versus B1 ($P = 0.389$), and A3 versus B3 ($P = 0.481$). *Post-hoc* pairwise comparison of mean post-cycle microhardness values is summarized in Table 3.

Discussion

The demand for natural products is increasing rapidly. Aloe vera is one of them, but there are very limited

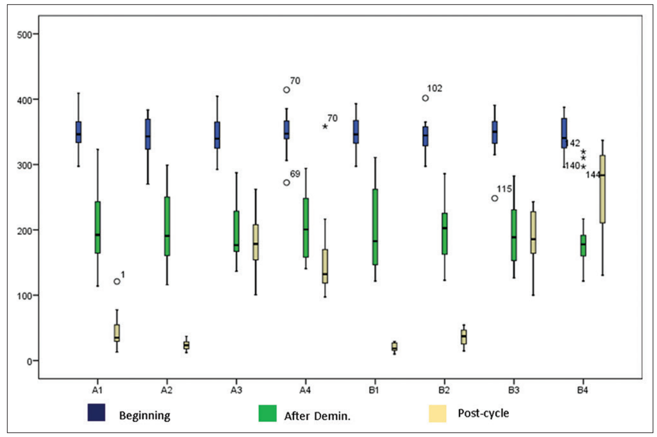

Figure 5: The comparison of the standard deviation and median values of all groups

studies in the literature on the remineralization efficiency of aloe vera.[24,25] In this study, it was aimed to evaluate the remineralization efficiency of commercially available toothpastes with different contents and pure aloe vera gel on demineralized enamel tissue by pH cycle method.

In studies using aloe vera, different techniques were used while obtaining aloe gel from the fresh plant. Jain, Supreet *et al*.[26] washed the aloe vera leaves, collected the aloe gel in a sterile container, and stored the gel at 4°C using dimethyl sulfoxide as the solvent. Carvalho *et al*.[27] scraped the aloe gel, wrapped it in a plastic film in a sterile collector, and kept it frozen at −18 to −25°C, followed by drying (lyophilization). In this study, similar to the work of Shende and Telrandhe[20] and Bhardwaj, Ballal, and Velmurugan,[21] aloe vera leaves were cut with a sterile knife and washed under running water to remove dust and debris. The leaves are cut with a sterile knife. The aloe gel was scraped, first cut into small pieces and crushed, homogenized, and applied. Different processes were not applied for storage and holding, as the aloe gel was applied immediately after obtaining and was prepared freshly before each application.

In intragroup comparisons, we can say that initial enamel lesion can be successfully created in all groups by measuring the values obtained after demineralization in all groups significantly lower than the initial values ($P < 0.001$).

Fluoride remains the gold standard for arresting caries lesions with multiple systematic reviews confirming the role of fluoride products in preventing dental caries.[28-32] In this study, there was no significant difference between 1100 ppm fluoride (Group A3) and 1000 ppm fluoride and aloe vera (Group B3) toothpaste groups after demineralization and post-cycle intragroup comparison ($P > 0.05$). This situation can be explained by the predominance of the demineralization procedure in the pH cycle under the *in-vitro* experimental conditions created in this study.

Fluoride-free groups (Groups A1, A2, B1, and B2) showed statistically significantly lower post-cycle values than all other groups. When the pH values of the groups were examined, they were measured in the range of pH: 6.1–6.5, pH: 5.5, pH: 6.5, and pH: 6.2, respectively. The critical pH of enamel tissue is generalized to 5.5, but this value is not fixed. It can vary depending on the type of acid in the environment, fluoride concentration, calcium, and phosphate ion concentration or mineral dissolution properties in different parts of the tooth.[33,34] In this study, it can be said that demineralization can be seen at higher pH values on tooth surfaces in a fluoride-free environment, due to the removal of the acid-resistant layer by applying surface treatments, and then the creation of artificial initial enamel lesions by applying demineralization to the samples. Additionally, considering that the demineralization process was predominate in the *in-vitro* pH cycle, it is possible to explain why statistically significantly lower post–pH cycle values were observed in the fluoride-free groups.

When the *post-hoc* pairwise comparison of the mean post-cycle microhardness values was examined, Group A3 showed statistically significant higher microhardness values than Group A2; Group B3 showed statistically significant higher microhardness values than Groups B2 and B4 than Group B3. In *in-vitro* caries models, a dose–response relationship was observed at different fluoride levels in inhibiting demineralization or promoting enamel remineralization.[35-37] It has been suggested that in the 1000–2500 ppm fluoride range, a cumulative 6% reduction in caries progress will be achieved with each additional 500 ppm fluoride over 1000 ppm fluoride.[38]

The fact that the mean microhardness value of Group A4 was lower than that of Group A3 does not match the expectation of dose–response relationship. In Walsh, Tanya *et al*.'s[36] review of different concentrations of fluoride toothpastes to prevent dental caries, although the general consensus is in favor of high fluoride products in the development of new caries in children and adolescents, the results of two studies evaluating the effect of fluoride toothpastes between 1000 and 1250 ppm and 1450 and 1500 ppm were in favor of lower fluoride toothpaste. The results of this systematic review support the conclusion that Group A3 showed higher mean microhardness than Group A4. However, the toothpastes used in the study are products of different commercial product. As a result, reductions in the microhardness values of the teeth may result from preparation/manufacturing process and the interaction of the components.

The results of this study supported the study of Al Haddad *et al*.[25] in which they investigated the remineralization efficiency of aloe vera, except for the group in which aloe vera gel was applied directly (Group B1). It has been found that toothpastes containing fluoride-free aloe vera have the least remineralization effect, which can be explained by the concentration of active ingredients found in lesser amounts in the toothpaste in a more saturated gel form and from the toothpaste production process. Differently in our study, we tried to imitate the natural process by applying the *in-vitro* pH cycle. Since the experimental materials were also applied between these cycles, it is possible to say that the pure aloe vera gels applied in the studies gave different results due to different *in-vitro* experimental conditions.

Silva *et al*.[24] aimed to evaluate the effects of tooth brushing on artificial white-spot lesions using fluoride toothpaste and aloe vera tooth gel. In the study, aloe vera–based tooth gel showed higher knoop microhardness values than 1450 ppm fluoride toothpaste. On the contrary, in our study, toothpaste containing non-fluoride aloe vera (Group B2) showed statistically significantly lower microhardness values than toothpaste containing 1450 ppm fluoride (Group A4). This may be due to the aloe vera concentration in the aloe vera–based toothpaste used in the study and the interaction of other ingredients in its content. Similarly, in the studies of Al-Haddad *et al*.,[25] toothpaste containing fluoride-free aloe vera showed lower microhardness values than 1450 ppm toothpaste.

While most toothpastes on the market contain NaF (sodium fluoride) or SMFP (sodium monofluorophosphate), SnF_2 (stannous fluoride) and AmF (amine fluoride) formulations are also available.[39] There are different amounts of NaF formulation in fluoride-containing toothpastes used in the study, and SMFP formulation in Group B4. This is very important in terms of evaluating the results. This is because Group B4 showed statistically significantly higher post-cycle microhardness values than all other groups, especially Group A4 with approximately the same fluoride content.

There used to be a controversy regarding the clinical efficacy obtained by NaF and SMFP toothpastes. Based on the premise that fluoride only exerts its effects on de- and remineralization as a free ion, several reports have claimed the superiority of NaF formulations (which releases free F^-), in comparison with SMFP, where fluoride is covalently bound to phosphate and requires enzymatic hydrolysis to release free F^-.[39]A meta-analysis by Johnson Mary F.[40] investigating the comparative effectiveness of NaF and SMFP toothpastes in preventing caries claimed a 6–7% efficacy difference in favor of NaF formulations. There are many studies showing that the caries prevention effect of NaF is better than SMFP.[41-43]

Toda and Featherstone[37] conducted an *in-vitro* pH cycle study to better understand the action mechanism of fluoride compound (NaF, AmF, and SMFP) in three different formulations, to investigate its anti-caries effectiveness on tooth enamel and to test the relationship between the effects of formulations on de/remineralization and free fluoride ion concentration. Commercially available toothpastes with different fluoride amounts and fluoride solutions of comparable concentrations were prepared in the study and SMFP-based toothpaste produced only a minor inhibitory effect on enamel lesion formation, similar to the 30-ppm NaF control solution.

In our study, contrary to these data, Group B4 showed statistically significant higher microhardness values than all other groups, despite the dominant demineralization process. This may be due to the synergistic interaction of aloe vera and SMFP formulation to promote remineralization. However, there were not enough data to support this interaction in the literature. Our study may be a pioneer in this respect. This result is very important in terms of guiding future *in-vitro* and *in-vivo* studies that will investigate the remineralization efficiency of aloe vera.

Fani and Kohanteb[44] investigated the inhibitory activities of aloe vera gel on some cariogenic (*Streptococcus mutans*), periodontopathic, and opportunistic periodontopathogen isolated from patients with dental caries and periodontal diseases. As a result of the study, it was concluded that the optimum concentration of aloe vera gel can be used as an antiseptic in the prevention of dental caries and periodontal diseases. Considering this result, it is predicted that toothpastes containing aloe vera may have a higher anti-caries effect in *in-vivo* conditions.

Our study has deficiencies such as the use of ingredients from different trademarks, uncertain aloe vera concentration, and the presence of different active ingredients. However, our study has strengths such as being one of the pioneering studies evaluating the remineralization efficiency of aloe vera, using different fluoride formulations, and trying to imitate nature with the pH cycle method.

In future studies, we recommend that different commercially available toothpastes containing aloe vera should be compared with different remineralization agents other than fluoride, and more importantly, experimental pastes should be prepared in order to investigate the synergistic effect of aloe vera and SMFP formulation, and comparative studies should be planned by eliminating the differences of commercially available toothpastes.

Conclusion

The use of toothpastes with fluoride is an effective method in the treatment of demineralized teeth. However, within the limits of this *in-vitro* study, it can be said that aloe vera increases the remineralization efficiency of toothpastes containing high-concentration fluoride.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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