



## INVESTIGATION OF THE CLINICAL AND MICROBIOLOGICAL EFFECTS OF DIFFERENT TOOTHPASTES: *IN-VIVO* STUDY

### ABSTRACT

**Objectives:** The purpose of this study is to compare the clinical, antibacterial and microbiological effects of the non-fluoride and fluoride toothpastes.

**Materials and Methods:** In this study eighty children (3 to 12 years old) were randomly divided into four groups and followed for four weeks. The first and second groups (40 children, 6-12 years) used different fluoride containing toothpastes; the third and fourth groups (40 children, 3-5 years) used non-fluoride toothpastes. The halitosis score, plaque index, gingival index, bleeding index, buffering capacities, *Mutans Streptococci*, *Lactobacilli* and yeast counts were recorded on 1<sup>st</sup> day, 7<sup>th</sup> day, 15<sup>th</sup> day and 30<sup>th</sup> day. The first and second groups; the third and fourth groups were compared with each other. Data were analyzed statistically by using Mann Whitney U tests, Wilcoxon Sign Test, Fisher Freeman Halton Exact Test and Mc Nemar Test with a significance level of  $p < 0.05$ .

**Results:** Statistically significant association was not found in the mean scores of halitosis, gingival index, plaque index, bleeding index, buffering capacity, *Mutans Streptococci*, *Lactobacilli* and yeast ( $p > 0.05$ ), between groups on the first day. All four toothpastes produced statistically significant reductions from 1st day to 30th days in scores of halitosis, plaque index, gingival index, bleeding index and buffering capacity ( $p < 0.01$ ;  $p < 0.05$ ), within groups. Statistically significant reductions were found according to in *Mutans Streptococci*, counts from 1st day to 30th day for group I, II and III ( $p < 0.05$ ); but was not found statistically significant changes in Group IV on the 30th days ( $p > 0.05$ ).

**Conclusions:** All tested toothpastes proved to be safe and significantly effective clinical and microbiological features.

**Key Words:** Child, fluoride, toothpaste, mutans streptococci, saliva.

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## INTRODUCTION

Periodontal diseases and dental caries are the two most common oral infections worldwide. Endogenous oral bacterial species and their metabolites play an important role in the initiation and progression of these infections.<sup>1,2</sup> Apart from this, caries is a multifactorial disease, so it can be affected by factors such as dental biofilm, sugar, host and time.<sup>3,4</sup> It is caused by impairment of the balance between the microflora in the mouth and the host biology.<sup>5</sup>

Acid-producing and tolerating microorganisms such as *Mutans Streptococci (MS)*, *Lactobacilli (LB)* and yeast, are seen as organisms that are responsible for the formation of caries.<sup>6,7</sup> Therefore, managing the mechanism of caries by controlling or removing the acidogenic bacteria has an important place in modern non-invasive treatment model of caries treatment with antibacterial approach.<sup>8,9</sup>

Biofilm control is an important procedure for the removal of microbial dental biofilm to prevent tooth decay and periodontal disease and to prevent the accumulation of teeth and adjacent gingival surfaces.<sup>6,10</sup>

It is thought that toothbrushing habit has a potential to removal dental biofilm and prevent caries with the fluoride toothpaste, one of these protective and preventive applications.<sup>11,12</sup>

Toothbrushing is one of the easiest individual practices used to ensure good oral hygiene. Regular toothbrushing habits that are effectively done with the selected toothpaste help to remove the dental plaque, one of the factors that play a role in the formation of tooth decay. Today, there are various toothbrushes and toothpastes, specially designed for children on the market.<sup>13,14</sup>

Toothpastes with antimicrobial effects have an important effect on the removal of both dental biofilm and gingivitis.<sup>15</sup> Studies on the use of antimicrobials in the prevention of caries have been going on for over 5 years.<sup>16,17</sup>

The fluoride-containing toothpastes are useful and the easiest way to maintain oral health by controlling the caries mechanism used with individual applications in the provision and development of oral hygiene.<sup>4,9,13,16</sup> Fluoride is to be an important source of material in the prevention and treatment of caries due to its cariostatic and remineralization properties.<sup>5,18,19,20</sup> Fluoride has a direct effect on MS biofilm formation, possibly due to the weakening of water-insoluble glucan production associated with the suppressed release of GtfB and GtfC from the bacterial cell membrane.<sup>21</sup>

The aimed of this study to investigate and compare the clinical and microbiological effects of sodium chlorite and fluoride-containing toothpastes *in-vivo*. The fluoride-containing toothpaste, the fluoride and sodium chlorite containing toothpaste, sodium chlorite containing and fluoride-free toothpaste were selected as the experimental groups. The null hypothesis of the study is that the efficacy of fluoride-containing toothpastes are better than the fluoride-free and chloride-containing toothpastes.

## MATERIALS AND METHODS

The study was approved by the Ethics Committee of the Istanbul University Faculty of Dentistry (2013/368) and was carried out in agreement with the Declaration of Helsinki principles. The informed contents were obtained from all participants and the study design followed CONSORT 2010 Statement: Updated guidelines for reporting parallel group randomized trials.<sup>22</sup>

All tested toothpastes and flow chart were shown in Table 1 and Figure 1.

**Table 1.** Materials used in this study

Groups	Product and manufacturer	Content
Group I	Sensodyne Pronamel (GlaxoSmithKline, USA)	1450 ppm NaF containing toothpaste
Group II	Oxyfresh (Oxyfresh, USA)	0.235% NaF and sodium chlorite containing toothpaste
Group III	Nenedent 2-4 (Dentinox, Berlin, Germany)	Fluoride- free toothpaste
Group IV	Oxyfresh (Oxyfresh, USA)	Fluoride-free, sodium chlorite- containing toothpaste

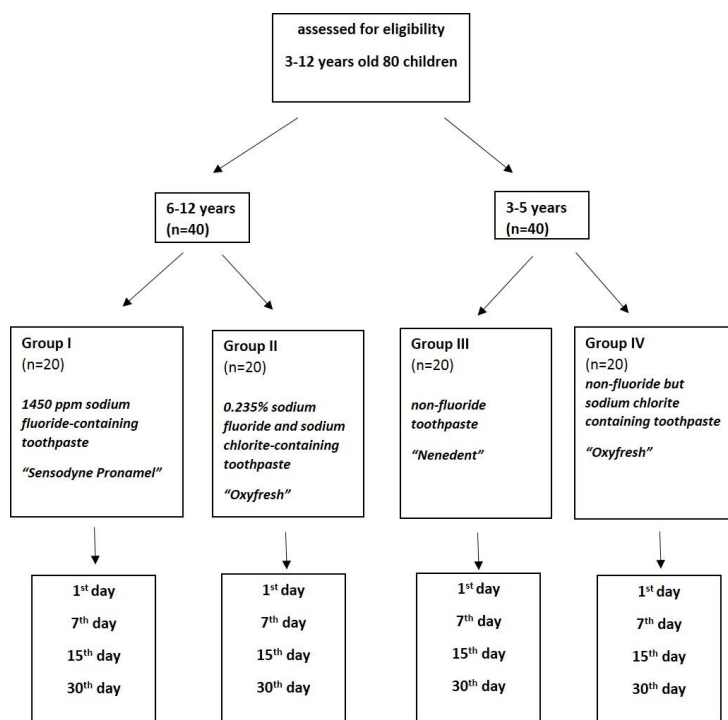


Figure 1. Flow chart of the study

The four commercially available toothpastes were: (1) 1450 ppm sodium fluoride-containing toothpaste (Sensodyne Pronamel 6+); (2) sodium chlorite and 0.235% sodium fluoride-containing toothpaste (Oxyfresh Toothpaste Fluoride); (3) nonfluoride toothpaste (Nenedent 2-4); and (4) nonfluoride toothpaste containing sodium chlorite (Oxyfresh Toothpaste Original). The study population was comprised of 80 healthy children who did not have any systemic problems, did not use regular medication, did not use antibiotics in the last 1 month, familiar with the habit of brushing teeth. 80 children (43 M, 37 F), 3-to 12-year-old (mean age  $7.51 \pm 2.24$ ) divided into four groups, were followed for four weeks. The first group (20 children, 6-12 years) used 1450 ppm sodium fluoride-containing toothpaste (Group I); the second group (20 children, 6-12 years) used 0.235% sodium fluoride and sodium chlorite-containing toothpaste (Group II); the third group (20 children, 3-5 years) used non-fluoride toothpaste (Group III) and the fourth group (20 children, 3-5 years) used non-fluoride but sodium chlorite containing toothpaste (Group IV). Initially, brushing frequency, decayed-missing-filled teeth were recorded clinically. The unstimulated saliva samples were collected in the morning hours and at least 2 hours after the last food or drink in sterile containers and analyzed

within one hour of collection. The Ericsson's method was used to measure the buffering capacity.<sup>23</sup> At the end of each quantitative culture phase, we determined the mean colony forming units (CFUs) for microbiological analysis. The halitosis score (Breath Checker TANITA Slim white HC-212S-WH), Silness & Loe plaque index, Silness & Loe gingival index, bleeding index, salivary buffering capacities, salivary *Mutans Streptococci*, *Lactobacilli* and yeast counts were recorded 1<sup>st</sup> day, 7<sup>th</sup> day, 15<sup>th</sup> day and 30<sup>th</sup> day. The first and second groups; the third and fourth groups were compared with each other.

The sample size calculation resulted in an 80% power at a 5% level of statistical significance and a 10% the difference between the groups, requiring 12 children for each group. In this study, we evaluated 20 children for each group. All measurements were performed by a single specialist (MK).

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS, IBM Corporation, Version 21.0; Armonk, NY, USA) software. The differences between the groups were statistically analyzed using the Mann Whitney U tests, Wilcoxon Sign Test, Fisher Freeman Halton Exact Test and Mc Nemar Test with a significance level of  $p < 0.05$ .

**RESULTS**

The mean DMFT (Decayed, Missing, Filled Tooth), DMFS (Decayed, Missing, Filled Surface), dft (decayed, filling tooth) and dfs (decayed, filling surface) are 0.8±1.54; 1.9±4.19; 4.45±3.36; 9±7.43 for 1st group and 1.75±1.92; 3.3±4.40; 4.65±2.91; 10.95±7.29 for 2nd group.

The mean df, dfs are 6.6±5.01; 11.9±8.70 for 3rd group and 8.4±2.98; 17.5±8.17 for 4th group. The mean scores of brushing frequency are in Table 2. No statistically significant difference was found between the brushing frequency and caries scores between the groups (p>0.05) (Table 2).

**Table 2.** Evaluation of brushing frequency, DMFT, DMFs, dft, dfs

	Group I	Group II	p	Group III	Group IV	p
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
<b>Brushing frequency</b>	0.8±0.77 (1)	1.2±1.5 (1)	<b>0.170</b>	1.35±0.88 (2)	1.5±0.61 (2)	<b>0.795</b>
<b>DMFT</b>	0.8±1.54 (0)	1.75±1.92 (1.5)	<b>0.068</b>	-	-	-
<b>DMFS</b>	1.9±4.19 (0)	3.3±4.40 (1.5)	<b>0.096</b>	-	-	-
<b>dft</b>	4.45±3.36(4)	4.65±2.91 (3.5)	<b>0.774</b>	6.6±5.01 (8)	8.4±2.98 (8)	<b>0.225</b>
<b>dfs</b>	9±7.43 (8)	10.95±7.29 (9)	<b>0.378</b>	11.9±8.70 (12)	17.5±8.17 (14.5)	<b>0.082</b>

*Mann Whitney U Test*

Statistically, significant association was not found the mean scores of halitosis, plaque index, gingival index, bleeding index, buffering capacity, MS, LB and yeast between the groups (p>0.05) (Table 3-10).

The mean scores of halitosis, plaque index, gingival index, bleeding index and buffering capacity were found to be statistically significantly decreased from day 1 to day 30 among all groups (p <0.01; p <0.05) (Table 3- 7).

**Table 3.** Evaluation of halitosis scores

Halitosis score	Group I	Group II	1p	Group III	Group IV	1p
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
<b>1st day</b>	0.45±0.83 (0)	0.8±0.70 (1)	<b>0.081</b>	0.8±0.77 (1)	1.3±0.92 (1)	<b>0.063</b>
<b>7th day</b>	0.25±0.44(0)	0.45±0.51 (0)	<b>0.190</b>	0.2±4.10 (0)	0.35±0.67 (0)	<b>0.603</b>
<b>15th day</b>	0±0 (0)	0±0 (0)	<b>1.000</b>	0.5±0.22 (0)	0.05±0.22 (0)	<b>1.000</b>
<b>30th day</b>	0±0 (0)	0±0 (0)	<b>1.000</b>	0±0 (0)	0±0 (0)	<b>1.000</b>
<b>1st-7th day 2p</b>	0.206	0.008**		0.001**	0.001**	
<b>1st-15th day 2p</b>	0.024*	0.001**		0.001**	0.001**	
<b>1st-30th day 2p</b>	0.024*	0.001**		0.001**	0.001**	

<sup>1</sup> Mann Whitney U Test

<sup>2</sup> Wilcoxon Sign Test

\*p<0.05

\*\*p<0.01

**Table 4.** Evaluation of plaque index

Plak index	Group I	Group II	1p	Group III	Group IV	1p
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
<b>1st day</b>	0.69±0.58 (0.75)	0.73±0.69 (0.5)	<b>0.935</b>	0.56±0.49 (0.5)	0.78±0.47 (0.75)	<b>0.073</b>
<b>7th day</b>	0.56±0.49 (0.5)	0.41±0.59 (0)	<b>0.200</b>	0.22±0.5 (0)	0.49±0.51 (0.5)	<b>0.076</b>
<b>15th day</b>	0.42±0.40 (0.5)	0.21±0.3 (0)	<b>0.070</b>	0.08±0.23 (0)	0.15±0.32 (0)	<b>0.604</b>
<b>30th day</b>	0.23±0.24 (0.12)	0.08±0.12 (0)	<b>0.048*</b>	0.01±0.06 (0)	0.01±0.06 (0)	<b>1.000</b>
<b>1st-7th day 2p</b>	0.011*	0.001**		0.001**	0.001**	
<b>1st-15th day 2p</b>	0.001**	0.001**		0.001**	0.001**	
<b>1st-30th day 2p</b>	0.001**	0.001**		0.001**	0.001**	

<sup>1</sup> Mann Whitney U Test

<sup>2</sup> Wilcoxon Sign Test

\*p<0.05

\*\*p<0.01

**Table 5.** Evaluation of gingival index

Gingival index	Group I	Group II	1p	Group III	Group IV	1p
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
<b>1st day</b>	0.36±0.45 (0.19)	0.38±0.39 (0.39)	<b>0.645</b>	0.23±0.21 (0.25)	0.57±0.27 (0.5)	<b>0.001**</b>
<b>7th day</b>	0.27±0.33 (0.08)	0.16±0.27 (0)	<b>0.299</b>	0.08±0.18 (0)	0.17±0.32 (0)	<b>0.393</b>
<b>15th day</b>	0.18±0.24 (0)	0.08±0.17 (0)	<b>0.090</b>	0.03±0.09 (0)	0.03±0.11 (0)	<b>0.594</b>
<b>30th day</b>	0.11±0.17 (0)	0.03±0.08 (0)	<b>0.065</b>	0.01±0.04 (0)	0.01±0.06 (0)	<b>0.594</b>
<b>1st-7th day 2p</b>	0.043*	0.001**		0.001**	0.001**	
<b>1st-15th day 2p</b>	0.005**	0.001**		0.001**	0.001**	
<b>1st-30th day 2p</b>	0.005**	0.001**		0.001**	0.001**	

<sup>1</sup> Mann Whitney U Test

<sup>2</sup> Wilcoxon Sign Test

\*p<0.05

\*\*p<0.01

Table 6. Evaluation of bleeding index

Bleeding index	Group I	Group II	<sup>1</sup> p	Group III	Group IV	<sup>1</sup> p
	Mean±SD	Mean±SD		Mean±SD	Mean±SD	
1st day	16.88±19.57 (18.8)	24.38±25.8 (25)	<b>0.421</b>	25.63±23.46 (25)	36.25±18.98 (25)	<b>0.042*</b>
7th day	15±15.5 (18.8)	12.5±18.58 (0)	<b>0.432</b>	12.5±20.28 (0)	11.25±20.64 (0)	<b>0.769</b>
15th day	8.13±13.62 (0)	3.75±8.21 (0)	<b>0.270</b>	6.88±15.95 (0)	2.5±11.18 (0)	<b>0.172</b>
30th day	2.5±6.54 (0)	1.88±6.12 (0)	<b>0.655</b>	3.13±7.98 (0)	1.25±5.59 (0)	<b>0.311</b>
1st-7th day <sup>2</sup> p	0.180	0.007**		0.001**	0.001**	
1st-15th day <sup>2</sup> p	0.004**	0.003**		0.001**	0.001**	
1st-30th day <sup>2</sup> p	0.003**	0.003**		0.001**	0.001**	

<sup>1</sup>Mann Whitney U Test

<sup>2</sup>Wilcoxon Sign Test

\*p<0.05

\*\*p<0.01

Table 7. Evaluation of buffering capacity

Buffering capacity		Group I	Group II	<sup>1</sup> p	Group III	Group IV	<sup>1</sup> p
		n (%)	n (%)		n (%)	n (%)	
1st day	High	3 (%15)	5 (%26.3)	<b>0.209</b>	5 (%27.8)	5 (%25)	<b>0.883</b>
	Medium	15 (%75)	9 (%47.4)		11 (%61.1)	14 (%70)	
	Low	2 (%10)	5 (%26.3)		2 (%11.1)	1 (%5)	
7th day	High	3 (%15.8)	1 (%5.9)	<b>0.507</b>	7 (%35)	4 (%20)	<b>0.651</b>
	Medium	12 (%63.2)	14 (%82.4)		11 (%55)	14 (%70)	
	Low	4 (%21.1)	2 (%11.8)		2 (%10)	2 (%10)	
15th day	High	1 (%5)	3 (%15.8)	<b>0.272</b>	3 (%15.8)	1 (%5.3)	<b>0.307</b>
	Medium	13 (%65)	14 (%73.7)		13 (%68.4)	17 (%89.5)	
	Low	6 (%30)	2 (%10.5)		3 (%15.8)	1 (%5.3)	
30th day	High	1 (%5)	2 (%10.5)	<b>0.865</b>	1 (%5.9)	3 (%15.8)	<b>0.605</b>
	Medium	16 (%80)	14 (%73.7)		16 (%94.1)	15 (%78.9)	
	Low	3 (%15)	3 (%15.8)		0 (%0)	1 (%5.3)	
1st-7th day <sup>2</sup> p		0.607	0.135		0.368	0.801	
1st-15th day <sup>2</sup> p		0.097	0.082		0.717	0.172	
1st-30th day <sup>2</sup> p		0.333	0.160		0.180	0.392	

<sup>1</sup>Fisher Freeman Halton Exact Test

<sup>2</sup>Mc Nemar Testi

There was a statistically significant decrease in the number of MS from day 1 to day 30 in Group I,II,III (p <0.05). But for Group IV, statistically

significant difference was not found in the number of MS on day 30 (p>0.05) (Table 8).

Table 8. Evaluation of MS counts

MS		Group I	Group II	<sup>1</sup> p	Group III	Group IV	<sup>1</sup> p
		n (%)	n (%)		n (%)	n (%)	
1st day	High	17 (%85)	15 (%75)	<b>0.695</b>	14 (%70)	14 (%70)	<b>0.488</b>
	Medium	3 (%15)	4 (%20)		4 (%20)	6 (%30)	
	Low	0 (%0)	1 (%5)		2 (%10)	0 (%0)	
7th day	High	11 (%55)	8 (%40)	<b>0.546</b>	14 (%70)	10 (%50)	<b>0.333</b>
	Medium	8 (%40)	9 (%45)		6 (%30)	9 (%45)	
	Low	1 (%5)	3 (%15)		0 (%0)	1 (%5)	
15th day	High	8 (%40)	8 (%40)	<b>1.000</b>	13 (%65)	11 (%55)	<b>0.719</b>
	Medium	10 (%50)	9 (%45)		6 (%30)	6 (%30)	
	Low	2 (%10)	3 (%15)		1 (%5)	3 (%15)	
30th day	High	7 (%35)	8 (%40)	<b>0.852</b>	13 (%65)	12 (%60)	<b>1,000</b>
	Medium	8 (%40)	6 (%30)		5 (%25)	6 (%30)	
	Low	5 (%25)	6 (%30)		2 (%10)	2 (%10)	
1st-7th day <sup>2</sup> p		0.014*	0.046*		0.458	0.025*	
1st-15th day <sup>2</sup> p		0.019*	0.046*		0.572	0.034*	
1st-30th day <sup>2</sup> p		0.019*	0.019*		0.072	0.102	

<sup>1</sup>Fisher Freeman Halton Exact Test

<sup>2</sup>Mc Nemar Testi

\*p<0.05

Statistically significant reductions were not found according to in LBand yeast counts from 1<sup>st</sup> day to 15<sup>th</sup> day, while statistically significant reduction

was found in the 30<sup>th</sup> day for the Group I (p<0.01). Statistically significant reductions were not found according to in LBand yeast count from

1<sup>st</sup> day to 30<sup>th</sup> day for group II, III and IV (p>0.05) (Table 9-10).

**Table 9.** Evaluation of LB counts

LB		Group I	Group II	<sup>1</sup> p	Group III	Group IV	<sup>1</sup> p
		n (%)	n (%)		n (%)	n (%)	
1st day	High	16 (%80)	9 (%45)	<b>0.066</b>	13 (%65)	13 (%65)	<b>0,175</b>
	Medium	3 (%15)	9 (%45)		7 (%35)	4 (%20)	
	Low	1 (%5)	2 (%10)		0 (%0)	3 (%15)	
7th day	High	14 (%70)	8 (%40)	<b>0.079</b>	10 (%50)	14 (%70)	<b>0,122</b>
	Medium	6 (%30)	9 (%45)		9 (%45)	3 (%15)	
	Low	0 (%0)	3 (%15)		1 (%5)	3 (%15)	
15th day	High	12 (%60)	5 (%25)	<b>0.082</b>	9 (%45)	12 (%60)	<b>0,543</b>
	Medium	6 (%30)	10 (%50)		9 (%45)	5 (%25)	
	Low	2 (%10)	5 (%25)		2 (%10)	3 (%15)	
30th day	High	4 (%20)	5 (%25)	<b>1.000</b>	8 (%40)	11 (%55)	<b>0,698</b>
	Medium	11 (%55)	11 (%55)		8 (%40)	6 (%30)	
	Low	5 (%25)	4 (%20)		4 (%20)	3 (%15)	
1st-7th day <sup>2</sup> p		0.564	0.513		0.223	0.317	
1st-15th day <sup>2</sup> p		0.135	0.055		0.115	0.572	
1st-30th day <sup>2</sup> p		0.003**	0.228		0.112	0.223	

<sup>1</sup> Fisher Freeman Halton Exact Test

<sup>2</sup> Mc Nemar Testi

\*p<0.05

**Table 10.** Evaluation of yeast counts

Yeast		Group I	Group II	<sup>1</sup> p	Group III	Group IV	<sup>1</sup> p
		n (%)	n (%)		n (%)	n (%)	
1st day	High	3 (%15)	0 (%0)	<b>0.251</b>	2 (%10)	1 (%5)	<b>0.378</b>
	Medium	10 (%50)	10 (%50)		10 (%50)	6 (%30)	
	Low	7 (%35)	10 (%50)		8 (%40)	13 (%65)	
7th day	High	2 (%10)	1 (%5)	<b>0.028*</b>	1 (%5)	0 (%0)	<b>0.748</b>
	Medium	12 (%60)	5 (%25)		8 (%40)	7 (%35)	
	Low	6 (%30)	14 (%70)		11 (%55)	13 (%65)	
15th day	High	2 (%10)	0 (%0)	<b>0.169</b>	0 (%0)	1 (%5)	<b>0.451</b>
	Medium	8 (%40)	5 (%25)		6 (%30)	3 (%15)	
	Low	10 (%50)	15 (%75)		14 (%70)	16 (%80)	
30th day	High	0 (%0)	0 (%0)	<b><sup>3</sup>0.479</b>	0 (%0)	1 (%5)	<b>0.176</b>
	Medium	7 (%35)	4 (%20)		9 (%45)	4 (%20)	
	Low	13 (%65)	16 (%80)		11 (%55)	15 (%75)	
1st-7th day <sup>2</sup> p		0.368	0.180		0.135	0.135	
1st-15th day <sup>2</sup> p		0.135	0.125		0.069	0.083	
1st-30th day <sup>2</sup> p		0.018*	0.070		0.572	0.317	

<sup>1</sup> Fisher Freeman Halton Exact Test <sup>3</sup> Continuity (Yates) Correction

<sup>2</sup>Mc Nemar Testi

\*p<0.05

## DISCUSSION

Fluoride toothpaste has been reported to be the most important treatment method to reduce the incidence of caries. Topically applied fluoride reduces enamel demineralization in the presence of bacterial plaque acid and then improves natural remineralization processes in the presence of salivary minerals.<sup>12,24</sup>

Several clinical trials have shown that sodium fluoride provides remineralization on demineralized white spot lesions.<sup>12,20</sup> However, based on various mechanisms, fluoride also exhibits some antibacterial and antifungal effects such as metabolic interference and reduction of dental plaque acidogenicity.<sup>10,15</sup>

In the meta-analysis studies of the preschool children, toothbrushing with fluoride toothpaste

significantly reduced the prevalence of caries scores in primary tooth decay.<sup>25,26,33,34,35,36,37,38,39,40</sup>

In our study, statistically significant difference was not found between groups according to brushing frequency. Also, there weren't any differences between groups according to caries scores.

Patil *et al.*<sup>27</sup> examined the effects of different oral hygiene practices on oral malodor *in-vivo* in 120 children aged between 7-15 years. Children were divided into groups according to 4 different oral hygiene categories (tooth brushing with fluoride-containing toothpaste, tongue cleaning, mouth rinsing, combination group). It had been shown that the combined group was even more effective when all oral hygiene procedures resulted in halitosis significantly reduced as a result of the study. In this study, statistically

significant association was not found between groups and the mean scores of halitosis.

Cagetti *et al.*<sup>28</sup> evaluated the effects of two different toothpastes in controlling supragingival dental plaque and bleeding on probing in 48 healthy schoolchildren aged between 8-10 years. The children were selected randomly and divided into two groups, using the two different toothpastes (experimental toothpaste group containing fluoride, triclosan, cetylpyridinium chloride, and essential oils, control toothpaste group containing fluoride without another antibacterial ingredient) twice a day for 2 minutes. There was no statistically significant difference between the two groups regarding bleeding on probing at the end of the 4-weeks, whereas the decrease in plaque index in the experimental group was found to be higher at the statistically significant level than the control group. In the present study, despite all of the toothpaste groups showed statistically significant differences in decreasing scores of halitosis, gingival index, plaque index, bleeding index, buffering capacity, no statistically significant differences were found in the mean scores of these parameters between groups.

In 2007, Magnusson *et al.*<sup>29</sup> found that the amount of MS decreased significantly after 6 months of use of triclosan, aminofluoride and stannous fluoride- containing toothpastes. In this study, it was found that all toothpastes with and without fluoride reduced the number of MS after 30 days.

Patil *et al.*<sup>11</sup> have shown the effects of fluoride-containing toothpastes on oral microorganisms, particularly on the reduction of MS in their study. All toothpastes used in the study have been reported to have antibacterial activity and it is stated that the presence of fluoride provides antimicrobial effects. In our study, there was a decrease in the number of MS in all fluoride and fluoride-free toothpastes. There was no statistically significant difference in the presence of fluoride in toothpastes that was effective in reducing the number of MS.

Carvalho *et al.*<sup>30</sup> evaluated the antimicrobial activity of the toothpastes *in-vitro*. Experimental toothpastes are chosen in cage-based, mango-based, fluoride-free and free of three fluorides, including extracts. As a result of the study, it has been reported that fluoride free toothpastes have inhibitory activity against MS and *Lactobacillus Acidophilus*.<sup>3,11,30</sup>

*In-vitro* studies have shown that the presence of fluoride at constant low concentration allows MS to produce less acid. Fluoride concentrates on the tooth plate, inhibiting carbohydrate metabolism. Thus, lactic acid production is reduced. At the same time, adhesive polysaccharides also affect the production of bacteria.<sup>31</sup> Studies have indicated that fluoride toothpaste results from the combined effect of fluoride-free components on a significant portion of the antimicrobial activity against MS.<sup>32</sup>

## CONCLUSIONS

Considering the limitations of this *in-vivo* study include the differences among individuals, salivary characteristics, and the differences between antimicrobial substances in saliva; the lack of control over the frequency and shape of brushing of participating children. At the beginning of this study, we informed that children should be brushed under the supervision of parents. In both age groups, we did not experience any problems with brushing and appointment timing. However, it should be kept in mind that personal skills may affect the results of the study.

In this study fluoride-free, chlorite-containing and fluoride-containing toothpastes were used. All tested toothpastes proved to be safe and significantly effective clinical and microbiological features.

Therefore, further clinical studies are needed to demonstrate the antimicrobial activity of toothpastes and to standardize differences.

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Meeting of the Canadian Association for Dental Research (CADR). March 11-14, 2015, Boston, Mass., USA, 2015. Seymen F, Koruyucu M, Topcuoglu N. Külekçi G. Investigation of the clinical and microbiological effects of different toothpastes.

### CONFLICTS OF INTEREST STATEMENT

The authors declare that there is no competing interest.

### **Farklı Diş Macunlarının Klinik ve Mikrobiyolojik Etkilerinin İncelenmesi: In Vivo Çalışma ÖZ**

**Amaç:** Bu çalışmanın amacı, florür içeren ve içermeyen diş macunlarının klinik, antibakteriyel ve mikrobiyolojik etkilerini karşılaştırmaktır. **Gereç ve Yöntemler:** Bu çalışmada 3-12 yaş arası 80 çocuk dört gruba ayrıldı ve dört hafta boyunca takip edildi. Birinci ve ikinci gruplar (40 çocuk, 6-12 yaş) farklı florürlü diş macunları kullandı; üçüncü ve dördüncü gruplar (40 çocuk, 3-5 yaş) florür içermeyen diş macunları kullandılar. Ağız kokusu skoru, plak indeksi, gingival indeks, kanama indeksi, tamponlama kapasiteleri, Mutans Streptokokları, Lactobacilli ve maya sayıları 1., 7., 15. ve 30. günde kaydedildi. Birinci ve ikinci grup; üçüncü ve dördüncü gruplar birbirleriyle karşılaştırıldı. Veriler istatistiksel olarak Mann Whitney U testi, Wilcoxon Sign Testi, Fisher Freeman Halton Exact Testi ve Mc Nemar Testi kullanılarak  $p < 0,05$  anlamlılık düzeyinde analiz edildi.

**Bulgular:** İlk gün; gruplar arasında, ağız kokusu, gingival indeks, plak indeksi, kanama indeksi, tamponlama kapasitesi, S Mutans, Lactobacilli ve maya ortalamaları arasında istatistiksel olarak anlamlı ilişki bulunmadı ( $p > 0,05$ ). Dört diş macununun her biri, gruplar arasında, 1. günden 30. güne ağız kokusu, plak indeksi, gingival indeks, kanama indeksi ve tamponlama kapasitesi skorlarında istatistiksel olarak anlamlı azalma sağlamıştır ( $p < 0,01$ ;  $p < 0,05$ ). Grup I, II ve III'te; S. Mutans değerleri 1. günden 30. güne kadar istatistiksel olarak azalma gösterirken ( $p < 0,05$ ); Grup IV'te 30. günde istatistiksel olarak anlamlı bir değişiklik izlenmemiştir ( $p > 0,05$ ). **Sonuç:** Test edilen tüm diş macunları güvenli ve anlamlı derecede etkili

klinik ve mikrobiyolojik özellikler göstermiştir.

**Anahtar Kelimeler:** Çocuk, florür, diş macunu, mutans streptokok, tükürük.

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