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# Comparison of Fluoride Uptake into Tooth Enamel Using Four Different Fluoride Varnishes

**Masumeh Moslemi[a], Samira Asghari Moghaddam[b], Parastoo Iranparvar[c] Fahimeh Kooshki[c], Solmaz Eskandarion[d]**

[a]Professor, Dept. of Pediatric Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[b]Assistant Professor, Dept. of Oral & Maxillofacial Radiology, Faculty of Dentistry, Zanjan University of Medical Sciences, Zanjan, Iran.
[c]Assistant Professor, Dept. of Pediatric Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[d] Researcher, Dept. of Dentistry & Oral Health, Section for Prosthetic Dentistry, Aarhus University, Aarhus, Denmark.
Correspondence to Samira Asghari Moghaddam (Email: samira.moghaddam110@gmail.com).
Correspondence to Parastoo Iranparvar (Email: prst.ip@gmail.com).
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**Objectives** The efficacy of fluoride varnish in preventing dental caries has been well documented. The objective of this study was to compare the impact of four different fluoride varnish brands containing 5% sodium fluoride on the sound enamel of permanent teeth.
**Methods** In this experimental study, 40 sound human premolar teeth were randomly assigned to four groups (Fluorilaq®, FluoroDose®, Preventa®, Kimia®; N=10). Each tooth was sectioned into buccal and lingual halves, corresponding to the control and the experimental subgroups, respectively. In the experimental halves, a semi-circular shaped area of the enamel was treated with the designated fluoride varnish. All specimens underwent Acid Etch Enamel Biopsy. Fluoride and Calcium concentrations were measured using a Potentiometer and Inductively Coupled Plasma optical emission spectrometer, respectively. The collected data were analyzed using paired t-test and one-way ANOVA at $P<0.05$.
**Results** There was a significant difference between the enamel fluoride content of experimental and control halves in all groups ($P<0.05$). Preventa® Fluoride varnish caused the highest Fluoride uptake, followed by FluoroDose®, Fluorilaq®, and Kimia®. All pair comparisons were statistically significant ($P<0.05$), except for Preventa® vs. FluoroDose® ($P=0.36$).
**Conclusion** Since all examined Fluoride varnishes increased the Fluoride content of the tooth enamel, they potentially qualify for caries prevention. Furthermore, Preventa® could be considered a good domestic alternative for other brands investigated in this research, in terms of enamel fluoride uptake.
**Keywords** Fluorides, Dental Enamel, Dental Caries, Tooth Remineralization

## Introduction

It is well established that professional topical fluoride treatment is one of the most effective methods to prevent dental caries in children and adults. [1, 2] Fluoride is effective in controlling dental caries by some mechanical actions, when present in dental plaque and saliva. Fluoride ions inhibit the demineralization of sound enamel and promote the remineralization of incipient lesions. Fluoride also inhibits dental caries by affecting the metabolic activity of cariogenic bacteria in the oral cavity. [3] Different fluoride products have been introduced to increase tooth enamel resistance against dental caries. [4] Fluoride solutions, gels, toothpaste, and mouthrinses have been used to deliver fluoride onto the tooth surface, with various levels of fluoride uptake and clinical efficiency. [5] Fluoride varnish is another fluoride product that is widely used in pediatric dentistry because of its positive characteristics such as easy handling and application for dentists and better acceptability to patients. [6] It is known to be especially beneficial in children with high caries risk. [7-10] Fluoride varnish is the only high-concentration topical fluoride product that can be used in preschoolers. It is shown to be effective in dental caries prevention of both primary and permanent teeth when used 2-4 times a year. [11] The efficacy of fluoride varnish in preventing dental caries has been documented in many studies, as a remineralization effect on caries-like lesions in enamel and even dentin. [12, 13] In a 36-month cluster randomized clinical trial, biannual fluoride varnish treatment in 6-7-year-old children effectively prevented dental caries in their first permanent molars. [14, 15] Compared to other fluoride products, fluoride varnish adheres to the tooth surface for a longer duration and interacts with the enamel over time. [16]

According to previous studies, different fluoride varnish brands might differ in their performance, in terms of enamel fluoride uptake and rehardening capabilities. [16] Al Dehailan et al. investigated the effect of five available Fluoride varnishes on early caries lesions and showed different abilities between fluoride varnishes to reharden and fluoridate these lesions. [12]

Considering the increasing popularity of fluoride varnish treatments in pediatric dentistry, and the need for evidence-based treatments in the daily practice of dentists [11], this in vitro study aimed to compare fluoride uptake into tooth enamel using four different fluoride varnishes. All the investigated fluoride varnishes were resin-based and contained 5% Sodium Fluoride (NaF). Among the methods for estimating the fluoride content of enamel, the acid etch biopsy method was chosen, having the benefits of lower cost and complexity, allowing direct measurement, and use of HCl as a safer etchant than perchloric acid. [17]

## Methods and Materials

This in-vitro experimental study was performed on 40 human premolar teeth following the proper institutional ethics approval (IR.SBMU.RIDS.REC.1395.268). The sample size was calculated according to the following formula to be 9 samples in each group:

$\frac{\overline{x_1}-\overline{x_2}}{SD}$ = 1.8 / α= 0.2 / β = 0.2 / k=4

The teeth were extracted for orthodontic purposes during the past three months and kept in normal saline solution at four degrees Celsius, which was renewed once per week. Considering the fact that hypoplastic lesions, cracks, and white spots interfere with fluoride uptake, all samples were examined by a stereomicroscope (SZX9; Olympus©, Tokyo, Japan) at ×10 magnification to select the teeth with sound enamel. [18, 19] Prior to the experiment, the selected teeth were cleaned by a medium manual toothbrush (Jordan©, Oslo, Norway) and deionized water for 30 seconds to remove debris from the surface. The teeth were randomly assigned into four groups to examine the effect of four NaF varnishes including Fluorilaq® (Pascal© Inc., Bellevue, USA), FluoroDose® (Centrix© Inc., Shelton, USA), Preventa® (Asia chimiTeb©, Tehran, Iran), and Kimia® (Kimia©, Tehran, Iran) (N=10 in each group). The characteristics of four fluoride varnishes investigated in the study are presented in Table 1.

**Table 1-** Characteristics of four fluoride varnishes investigated in the study

| Product Name | Manufacturer | Fluoride source & concentration | Carrier | Other active ingredient | Package |
|---|---|---|---|---|---|
| Preventa® | Asia chimi Teb Co., Tehran, Iran | NaF 5% | Resin | Xanthan | Single-use (0.5 ml) |
| FluoroDose® | Centrix Inc., Shelton, USA | NaF 5% | Resin | - | Single-use (0.3 ml) LolliPack®package |
| Fluorilaq® | Pascal Co Inc., Bellevue, USA | NaF 5% | Resin | Xylitol | Single-use (0.4 ml) /10 ml tube |
| Kimia® | Kimia Co., Tehran, Iran | NaF 5% | Modified resin | - | 2.5 ml syringe |

The roots of all selected teeth were cut at two millimeters below the cementoenamel junction and discarded. In order to avoid the bias resulting from differences in baseline fluoride concentration of samples, each tooth was sectioned into two identical buccal and lingual halves along the long axis using a diamond disk (TDV®, Pomerode, Santa Catarina, Brazil) on a straight high-speed dental handpiece with cooling water. The buccal half of each tooth was used as the control and the lingual half was used for the experiment. After being washed with deionized water, the prepared specimens were stored at room temperature until they were completely dry.

Circle-shaped pieces of adhesive tape with a diameter of six millimeters (mm) were cut out using a punch machine and divided into two semi-circles using a precise engineering ruler. [20-22] Subsequently, each semi-circle adhesive piece was pasted to the central area of each specimen and burnished carefully onto the tooth surface until becoming fully attached. These stickers outlined the area of the tooth under biopsy. The remaining surface of each specimen was then carefully coated with two layers of acid-resistant nail polish (Maybelline©, L'Oréal, New York City, U.S). After becoming dried out, windows of pre-defined surface enamel were obtained by removing semi-circle adhesives. The enamel windows on lingual halves of samples in each group were treated with the designated fluoride varnish, which was applied twice at a one-minute interval. Thereafter, the samples were left untouched at room temperature for four minutes and placed in a magnetic stirrer (RH Basic2, IKA©, Germany) with a speed of 100 revolutions per minute (rpm) for one hour. Finally, all samples were kept in artificial saliva at 37 $^0$C for 24 hours.

In order to measure the amount of fluoride, all samples underwent an Acid Etch Enamel Biopsy. Eighty cryotubes were washed with deionized water, dried, and coded by someone blinded to the process. Each specimen was etched for 30 seconds in a cryotube containing one milliliter (mL) of perchloric acid with a concentration of 0.5 Molar (M). After etching, enamel window surfaces were washed with two mL of 0.2 M KOH solution in the same cryotube. [21, 22] Enamel surfaces were immediately wiped dry with a cotton pellet and returned to the cryotube. Fluoride and Calcium concentrations in the biopsy solution were determined using a Potentiometer (GDM-356, GWInstek©, Taiwan) and Optical Emission Spectrometer (Optima 8000, PerkinElmer©, MA, USA), respectively.

It should be mentioned here that the fluoride measurement in the biopsy solution does not directly determine the fluoride concentration in the enamel. In order to obtain the concentration of fluoride in enamel ($C_{FT}$), the ratio of the fluoride weight ($W_F$) to the weight of dissolved enamel ($W_E$) in each sample must be calculated and converted to ppm. In the Acid Etch Enamel Biopsy method, it is not possible to weigh the dissolved enamel directly. Since enamel contains 37.4% calcium, the dissolved enamel weight ($W_E$) can be obtained using the calcium weight in the biopsy solution ($W_{Ca}$). The weight of calcium ($W_{Ca}$) in

the solution can also be calculated according to its concentration (Ca), which is determined in ppm in the experiment. The following formulas were used to calculate the final fluoride concentration in enamel samples. Concentrations (C) are in ppm and weights are in micrograms (µg).

$W_{Ca} = C_{(Ca)} \times 3$ $W_F = C_{(F)} \times 3$

$W_E = W_{Ca} \times (100 / 37.4)$ $C_{FT} = (W_F \times 10^6) / W_E$

The following formula was used to determine the depth of the biopsy:

$$\text{Depth of the Biopsy} = \frac{\text{Dissolved Enamel Weight; } W_E\ (\mu g)}{\text{Area of the Biopsy } (mm^2) \times \text{Density of Enamel } \left(\frac{gr}{cm^3}\right)}$$

The density of enamel is 2.95 gr/cm$^3$, therefore the enamel weight and the depth of each etched layer could be determined based on the area, which is a semi-circle with a diameter of 6 mm, equal to 14.13 mm$^2$. [18, 23]

Statistical analysis was performed using SPSS 22.0 software (IBM©, Chicago, Ill., USA) (α equals 0.05). The depth of biopsy layer and fluoride concentration in each group was evaluated using Kolmogorov-Smirnov test, and due to the normality of distribution, the paired t-test was used to statistically compare the experimental and control halves in each test group. One-way analysis of variance (ANOVA) was carried out to compare the fluoride uptake among four groups.

## Results

According to the one-way ANOVA test, the fluoride concentration and enamel biopsy layer depth were not significantly different between the control sub-groups (P=0.54). The results of enamel biopsy layer depth and fluoride concentration are shown in Tables 2 and 3. All the fluoride varnishes increased the fluoride content of the experimental halves compared to the control halves. Paired t-test showed significant differences between experimental and control halves in all groups (P<0.05). The results showed that Preventa® Fluoride varnish caused the highest Fluoride uptake, followed by FluoroDose®, Fluorilaq®, and Kimia®. According to the One-way ANOVA test, a significant difference was observed between all groups, except between Preventa® and Flurodose® (P=0.36) (Table 4). The raw statistical data is included in the supplementary material (Suppl. 1).

**Table 2**. Enamel biopsy layer depth in experimental and control groups

| Fluoride varnish brand | Enamel biopsy layer depth in control halves (µm) | Enamel biopsy layer depth in experimental halves (µm) | P-value |
|---|---|---|---|
| Fluorilaq® | 16.42 ± 5.56 | 11.55 ± 4.06 | 0.004 |
| Fluorodose® | 19.97 ± 3.45 | 13.45 ± 4.32 | 0.004 |
| Preventa® | 19.2 ± 5.02 | 9.45 ± 5.33 | <0.001 |
| Kimia® | 18.63 ± 3.58 | 7.99 ± 1.68 | <0.001 |

* Significance level=0.05, at a confidence interval of 95%, according to the Paired t-test.

**Table 3.** Mean±SD of fluoride concentrations of enamel samples in experimental and control groups.

| Fluoride varnish brand | Fluoride concentration in control halves | Fluoride concentration in experimental halves | P- value |
|---|---|---|---|
| Fluorilaq® | 961.32 ± 468.35 | 6026.72 ± 1831.99 | <0.001 |
| Fluorodose® | 861.16 ± 300 | 14905.1 ± 6816.13 | <0.001 |
| Preventa® | 949.9 ± 397.04 | 27070.39 ± 21209.27 | 0.003 |
| Kimia® | 754.08 ± 190.48 | 2882.79 ± 925.66 | <0.001 |

* Significance level=0.05, at a confidence interval of 95%, according to the Paired t-test.

**Table 4.** P-values measurements for paired comparisons of fluoride uptake among the experimental groups following the application of four different fluoride varnish brands.

| Groups | Kimia®vs Fluorilaq® | Preventa®vs Fluorilaq® | FluoroDose®vs Fluorilaq® | Kimia®vs FluoroDose® | Preventa®vs FluoroDose® | Kimia®vs Preventa® |
|---|---|---|---|---|---|---|
| P value | 0.003 | 0.049 | 0.018 | 0.003 | 0.36 | 0.024 |

* Significance level=0.05, at a confidence interval of 95%, according to one-way analysis of variance.

## Discussion

Determination of the fluoride uptake into the enamel is one of the methods available to determine the performance of topical fluoride products. Although the amount of fluoride uptake is not always a definite indicator to evaluate the clinical efficacy in inhibiting caries, it is one of the most common laboratory tests being used. [24]

In the present study, the uptake of fluoride by enamel structure following the application of four different fluoride varnish products containing 5% sodium fluoride was investigated. The results showed an increase in enamel

fluoride content for all the specimens exposed to the fluoride varnish. Preventa® Fluoride varnish caused the highest Fluoride uptake, followed by FluoroDose®, Fluorilaq®, and Kimia®. Since the difference between Preventa® fluoride varnish and FluoroDose® was not statistically significant, Preventa® could be considered as a good domestic alternative for other fluoride varnish brands investigated in this research, in terms of enamel fluoride uptake.

Various research has already been conducted to compare the impact of topical fluoride products including mouthrinses, toothpastes, gels, and varnishes of different brands. Al Dehailan et al. [12] in an in vitro study investigated the effect of five commercially available fluoride varnishes on caries lesions by evaluating the fluoride uptake, fluoride rehardening capability, and fluoride release elements. The result showed that there was no significant difference among the products regarding the fluoride uptake; though, they had a different impact in terms of the rehardening capability, and total fluoride release. In a recent study by Kavya et al., the anticaries efficacy of three different fluoride varnish brands was evaluated using a scanning electron microscope, and a statistically significant difference was observed between them. [16] In another study, Schemehorn et al. [4] compared two different sodium fluoride varnishes with different sources of calcium and phosphate. They demonstrated that the fluoride varnish containing tri-calcium phosphate (TCP) delivers significantly less fluoride to both the sound and demineralized enamel compared to amorphous calcium phosphate (ACP)-containing varnish.

Abdoli et al [25] compared the enamel fluoride uptake by Ariadent® (re-branded as Preventa®) and Sultan fluoride varnishes. They confirmed the fact that the domestic product could be considered a good alternative to similar foreign products, which was in line with the results of the present study. In another study, Preventa® fluoride varnish significantly increased the enamel microhardness score to levels comparable with Duraflor varnish. [26] Although in the present study the enamel fluoride uptake using Preventa® fluoride varnish was shown to be promising, it failed to show any antibacterial properties against Streptococcus Mutans in a study by Jafari et al. [27]

Many studies have evaluated various domestic topical fluoride products in terms of enamel fluoride uptake. In a study by Ansari et al [21], Iranian fluoride mouthrinse and NaF rinses were compared. The result showed no significant difference between the levels of fluoride uptake among the products in two layers of enamel biopsy. However, Poureslami et al [28], reported that 0.4% stannous fluoride gel (Sultan. Co) resulted in a higher fluoride uptake into the tooth enamel compared to 0.05% sodium fluoride gel (domestic products), which was attributed to its higher amount of fluoride ion. Navabi, et al compared the enamel fluoride uptake by two different fluoride products and reported that Duraflor varnish caused higher fluoride uptake compared to APF fluoride gel. [20] Moreover, another study showed that Fluoride uptake is lower in Pooneh pediatric toothpaste compared to the tested ADA-approved dentifrice in sound enamel of primary teeth. [18]

One of the factors causing such differences in fluoride uptake by using various products is the presence of metal ions such as Calcium or Aluminum surrounded or combined with fluoride ions, inhibiting the activity of fluoride ions. [22] Therefore, the presence of each compound such as colors, flavors, and metal ions in the composition of fluoride varnish could weaken the effectiveness of fluoride ions. Since the exact formulation of the investigated fluoride varnishes has not been announced in detail by the manufacturers, a definite conclusion cannot be drawn regarding the exact reason behind the differences in Fluoride uptake amount. The limitation of the present study was the in-vitro design, overlooking the impact of the oral environment, including the confounders such as salivary elements. Therefore, future studies in a clinical setting are required to draw more definitive conclusions.

## Conclusion

The enamel fluoride uptake by Preventa® fluoride varnish was not significantly different from FluoroDose; therefore, Preventa® could be considered a good domestic alternative for other brands investigated in this research, in terms of enamel fluoride uptake.

**Supplementary Materials:** None

**Author Contributions:**

Masumeh Moslemi: study concept, study design, experimental procedure, review;
Samira Asghari moghaddam: study design, literature review, experimental procedures, statistical analysis
Parastoo Iranparvar: literature review, statistical analysis, manuscript preparation, editing and review
Fahimeh Kooshki: study concept, study design, experimental procedure, review
Solmaz Eskandarion: literature review, statistical analysis, editing and review

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**Informed Consent Statement:** The present study was conducted after being approved by the committee for ethics in research, school of dentistry, Shahid Beheshti University of Medical Sciences (IR.SBMU.RIDS.REC.1395.268). The study design was in accordance with the Helsinki

Declaration of Human Rights. The teeth were extracted for orthodontic purposes and were included in the study after obtaining the written informed consent document.

**Data Availability Statement:** All data generated or analyzed during this study are included in this published article.

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A preprint has previously been published in Research Square (https://www.researchsquare.com/article/rs-1905348/v1). [29]

**Conflict of Interest**

No Conflict of Interest Declared ■

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