



Salivary Fluoride Retention: A Comparative Analysis between Fluoride Containing Chewing Sticks and a Non Herbal Fluoridated Toothpaste

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ABSTRACT

Aim: To determine and compare salivary fluoride retention after the use of different fluoride-containing chewing sticks and a non-herbal fluoridated toothpaste.

Materials and methods: This double-blind cross-over experimental study was undertaken among twenty randomly selected senior secondary students in Ibadan, Nigeria. Saliva samples were collected to establish baseline fluoride concentration before the use of chewing sticks and non-herbal fluoridated toothpaste. Four commonly used chewing sticks and one non-herbal fluoridated toothpaste were each used at two days interval, and saliva samples were collected at 0, 10, 30, 45 and 60 minutes after each use. These samples were stored and transported in Gio'Style coolers to the laboratory, where they were analyzed for fluoride concentration using a spectrophotometer at a wavelength of 620 nm. Data were analyzed using descriptive and inferential statistics at $p < 0.05$.

Result: At baseline mean (\pm SD) salivary fluoride concentration of participants was 25.95 (\pm 4.58) ppm. The mean (\pm SD) salivary fluoride concentration at 0 minutes was 228.0 (\pm 032.80) ppm, 427.65 (\pm 122.85) ppm, 413.45 (\pm 78.08) ppm, 329.05 (\pm 83.12) ppm and 323.80 (\pm 66.41) ppm corresponding to Alchornea laxiflora, Zanthoxylum zanthoxyloides, Anogeissus leocarpus, Masularia acuminata and non-herbal toothpaste respectively. At 60 minutes Zanthoxylum zanthoxyloides had the highest mean (\pm SD) fluoride concentration of 44.75 (\pm 13.32) ppm. The differences in mean (\pm SD) salivary fluoride

concentrations amongst these tooth cleaning aids at 60 minutes were statistically significant ($p < 0.001$).

Conclusion: Zanthoxylum zanthoxyloides had the highest mean (\pm SD) salivary fluoride retention followed by the non-herbal fluoridated toothpaste.

Clinical significance: The use of chewing sticks can be a cost-effective and efficient means of caries prevention if used properly at regular interval.

Keywords: Caries, Chewing sticks, Non-herbal fluoride toothpaste, Salivary fluoride.

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INTRODUCTION

Dental caries is an infectious multifactorial disease of the erupted tooth. It is the most prevalent oral disease of childhood and also affects the vast majority of adults worldwide.^{1,2} The prevalence of dental caries is on the increase in developing countries due to increased consumption of refined sugar and inadequate use of fluoridated products.^{3,4} This increase in prevalence is more in rural areas because of accessibility to cariogenic diet and other risk factors.^{5,6} Caries prevalence has reduced in developed countries due to the use of fluoridated materials.⁷

Fluoride increases the resistance of the teeth to dental caries acting mainly by its topical application.⁸ The use of fluoridated toothpaste is the most popular means of topical fluoride application and saliva acts as a carrier for the fluoride.^{9,10} The presence of fluoride in saliva after the use of fluoride-containing materials can prevent dental caries formation and also reverse incipient caries.¹¹

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Fluoride is found naturally in some chewing sticks,¹² and the use of chewing sticks as an oral cleaning tool is common in many parts of the world.^{12,13} Chewing sticks contain compounds that have hemostatic, analgesic, antimicrobial, buffer and antiplaque effects thereby demonstrating their chemical function.¹³

Lack of proper exposure to fluoride in developing countries may have resulted in an increased prevalence of dental caries, and also low socio-economic status has been implicated as a risk factor to the development of dental caries.^{14,15}

The cost of commercial fluoridated material and their financial burden on low-income earners makes chewing sticks a viable alternative in providing the required fluoride in poor communities. As such World Health Organization has recommended the use of chewing sticks as an effective tool for oral hygiene in areas where they are used normally.¹⁶ Optimum oral health can thus be attained by using chewing sticks, but this depends on its regular use with proper and effective techniques.¹⁷

The aim of this study was to determine and compare salivary fluoride retention over time after the use of different fluoride-containing chewing sticks and a non-herbal fluoridated toothpaste.

MATERIALS AND METHODS

The study was undertaken according to the guidelines in the Declaration of Helsinki on research on human subjects and the study protocol was approved by the University of Ibadan/University College Hospital Ibadan Nigeria Ethical Review Board (UI/EC/15/0117).

- *Four chewing sticks: Alchornea laxiflora, Zanthoxylum zanthoxyloides, Anogeissus leocarpus and Masularia acuminata* were randomly selected from a list of commonly used chewing sticks whose fluoride concentrations were determined by Atomic absorption spectrophotometry method in a previous study.¹² The fluoride concentration of *Alchornea laxiflora*, *Zanthoxylum zanthoxyloides*, *Anogeissus leocarpus* and *Masularia acuminata* as reported in the study was 347 ppm, 1845 ppm, 383 ppm, and 365 ppm, respectively.¹² These four chewing sticks containing fluoride naturally were obtained from the Forest Research Institute of Nigeria, Ibadan, Oyo State Nigeria. The pictorial representation of these chewing stick is seen in Figure 1. A list of all non-herbal fluoridated toothpaste available in the market was drawn, and one of the toothpastes (Close-up toothpaste) was randomly selected by balloting from



Figs 1A to D: Fluoride containing chewing sticks (A) *Alchornea laxiflora*; (B) *Zanthoxylum zanthoxyloides*; (C) *Anogeissus leocarpus*; (D) *Masularia acuminata*

the list. This toothpaste was then purchased in a corner shop and kept on the bench in the fluoride research laboratory of Institute of Agricultural Research and Training (IAR and T) Ibadan, Nigeria.

A public secondary school was randomly selected from a list of public schools in Ibadan. Thirty secondary students were randomly selected by balloting from the schools' admission register provided by the head of school. Consent to participate in the study was obtained from the legal guardians of the students. Among the selected students, twenty who met the inclusion criteria were, caries free and had- fair to good oral hygiene, not wearing any dental prosthesis and with a stimulated salivary flow rate of between 0.8 mL/min and 2 mL/min were included in the study. The stimulated salivary flow rate was determined for each student after chewing paraffin tape for five minutes, and immediately saliva was produced it was collected by the aid of a funnel, in well-labeled graduated plastic bottles. The saliva samples in the bottle were placed in a rack, and after the disappearance of salivary froth, disposable 5ml sterile syringes were used to dispense and measure the saliva produced by each participant. To determine the salivary flow rate of each student, individually produced saliva in milliliters was divided by 5 minutes the total time of chewing the paraffin tape. The salivary flow rate of each participant was determined two days before determining baseline salivary fluoride concentration.

Baseline saliva was collected from the 20 study participants by telling them to gently spit into well-labeled graduated plastic bottles with the aid of funnels, and this was done a day before commencing the use of chewing sticks and non-herbal fluoride toothpaste. Before saliva collection, the students were told not to use chewing sticks, and any other fluoride-containing a product such as toothpaste or mouth rinse a day before and on the day of sample collection. To prevent contamination of samples by food debris, the students were also told not to eat anything before and in between sample collection. Each chewing stick was used for 4 minutes, 2 minutes for chewing stick and 2 minutes for cleaning the surface of the teeth with the tuft of the chewed stick.

The non-herbal toothpaste and toothbrush were also used for teeth cleaning for 4 minutes. The participant rinsed with 10 mL of deionized water for 10 seconds after use of each chewing stick and the non-herbal fluoride toothpaste before saliva samples were collected.

Each of the four chewing stick and the non-herbal fluoride toothpaste were used by the 20 participants on different days at two days interval. Saliva samples were collected at timed intervals of 0, 10, 30, 45 and 60 minutes from each participant into a labeled container.

Each time the saliva samples were collected, they were kept and transported in a Gio'Style cooler containing ice

packs to the fluoride research laboratory in the Institute of Agricultural Research and Training (IAR and T) immediately after collection.

On arrival at the laboratory, the saliva samples were removed from the Gio'Style cooler and allowed to stand on a rack for 20 minutes at room temperature. Then 5 mL of each saliva sample was transferred into a well-labeled centrifuge tube using a pipette, and 10 mm of 6N NaOH plus 10 mL of 1N Acetic acid were then added to the well-labeled centrifuge tube. These mixtures were centrifuged at 3000 RPM for 30 minutes in an 800 D centrifuge (Searchtech instrument Jiangsu, China). The supernatant was decanted into a clean 2 mL tests tube and was analyzed for fluoride.

Samples of fluoridated toothpaste weighing 100 mg were dispensed from the non-herbal fluoridated toothpaste into a beaker, and 10.0 mL of deionized water was added to it and stirred using a magnetic stirring machine (JHB-LABTECH, JHB-SH3, Germany) until a homogenous mix was obtained. Then 5 mL of the homogenous mix of non-herbal fluoridated toothpaste was transferred into a labeled centrifuge tube, and 10 mL of 6N NaOH and 10 mL of 1N Acetic acid were added. This solution was thoroughly mixed using a magnetic stirring machine (JHB-LABTECH, JHB-SH3, Germany) and loaded into a centrifuge machine (Searchtech instrument Jiangsu, China) and centrifuged at 3000RPM for 30 minutes. After removal from the centrifuge machine, the supernatant was decanted into a clean 2 mL test tube for fluoride analysis.

Fluoride Analysis

Before sample analysis, a fluoride calibration curve was plotted for standardization. This was a plot of absorbance against concentration for a series of diluted standard solutions whose concentrations were known. The absorbance readings of the known fluoride standard solutions were obtained using a spectrophotometer (Model: Spectronic 21D, Serial number: 0817783, Germany) at a wavelength of 620 nm. The plotted fluoride calibration curve was then used to determine the unknown fluoride concentrations in the saliva samples. The values obtained were converted to parts per million using a fluoride conversion chart.

Data Handling and Statistical Analysis

The data were entered into statistical package for social sciences (SPSS Inc. Chicago IL) version 19 and later mean (\pm SD) were generated. To ensure the validity of data entry; the data were entered twice and compared. Descriptive statistics such as frequency, proportions, means and standard deviations were generated for relevant variables. Student t-test was used to compare the means

of two groups while the analysis of variance was used to compare means of more than two groups. Pearson's correlation was used to determine the relationship between an independent and dependent variable. The level of significance was set at $p < 0.05$.

RESULTS

The study participants consisted of 11 males and 9 females with an age range of 14–17 years and their mean (\pm SD) age of 15.15 (± 0.81) years. The salivary flow rate of participants ranged between 0.80 and 1.29 mL/min with a mean (\pm SD) salivary flow rate of 1.08 (± 0.21) mL/min. The mean (\pm SD) salivary flow rate was 1.13 (± 0.24) mL/min and 1.04 (± 0.18) mL/min in females and males respectively ($p = 0.36$).

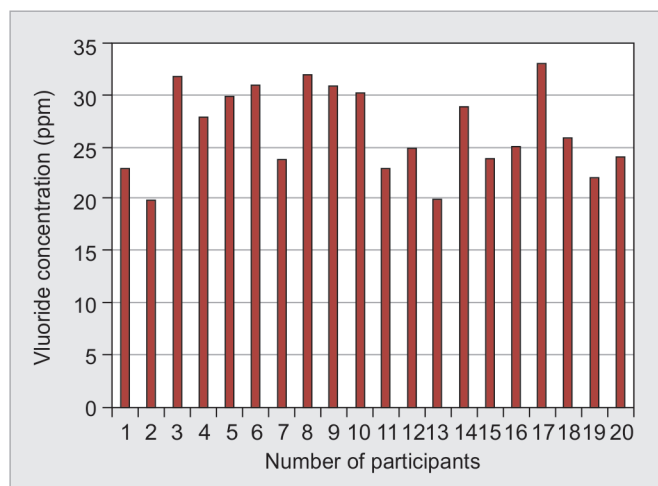
The baseline salivary fluoride concentration of participants as shown in Graph 1, it ranged from 18 to 33 ppm with a mean (\pm SD) of 25.95 (± 4.58) ppm. The mean (\pm SD) baseline salivary fluoride concentration of males was 27.00 (± 6.59) ppm and females 26.56 (± 4.16) ppm ($p = 0.86$).

Graph 2 illustrates the mean (\pm SD) salivary fluoride concentration before and after the use of chewing sticks and non-herbal toothpaste at different time intervals.

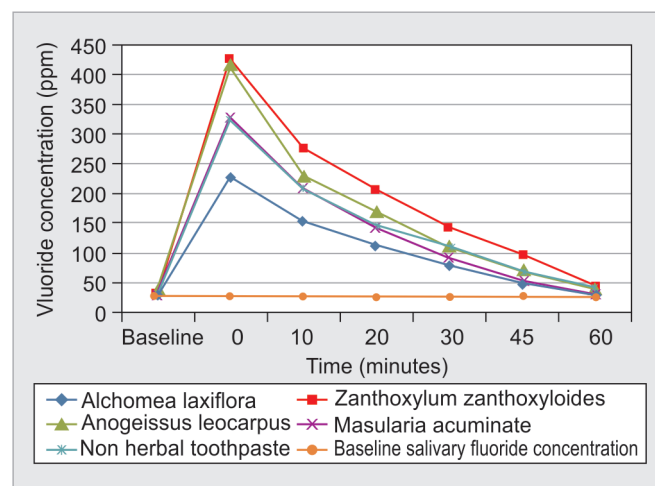
Zanthoxylum zanthoxyloides had the highest mean (\pm SD) salivary fluoride concentration at all timed intervals. The mean (\pm SD) salivary fluoride concentration of *Masularia acuminata* 329.05 (± 83.12) ppm and the non-herbal toothpaste 323.80 (± 66.41) ppm were similar at zero minutes. At 60 minutes interval, the mean (\pm SD) salivary fluoride concentration of the chewing sticks and non-herbal fluoridated toothpaste were higher than the mean (\pm SD) baseline salivary fluoride concentration. The chewing stick *Zanthoxylum zanthoxyloides* had the highest mean (\pm SD) salivary fluoride concentration of 44.75 (± 13.32) ppm at 60 minutes, followed by the non-herbal fluoridated toothpaste with a mean (\pm SD) of 42.70 (± 21.24) ppm. The difference between mean (\pm SD) salivary fluoride concentration after the use of chewing sticks and non-herbal toothpaste at the various timed interval was statistically significant with $p = 0.001$ (Table 1).

DISCUSSION

Over the last decade, there has been a marked decline in the incidence of dental caries in many developed



Graph 1: Baseline salivary fluoride concentrations of participants (n = 20)



Graph 2: Mean (\pm SD) salivary fluoride concentration after the use of different chewing sticks and non-herbal toothpaste over time

Table 1: Mean (\pm SD) salivary fluoride concentration at timed intervals after use of chewing sticks and non-herbal toothpaste

Time intervals (min)	Chewing sticks					Post hoc test
	<i>Alchornea laxiflora</i> mean (\pm SD)	<i>Zanthoxylum zanthoxyloides</i> mean (\pm SD)	<i>Anogeissus leocarpus</i> mean (\pm SD)	<i>Masularia acuminata</i> mean (\pm SD)	Non-herbal toothpaste mean (\pm SD)	
0	228.00 (± 32.81) ^{a,b,c,d}	427.65 (± 122.85) ^{a,e}	413.45 (± 78.08) ^{b,f,g}	329.05 (± 83.12) ^{c,f}	323.80 (± 66.41) ^{d,e,g}	a,b,c,d,e,f,g $p = 0.001$
10	152.93 (± 36.89) ^{a,b,c,d}	274.35 (± 61.85) ^{a,e,f,g}	226.55 (± 43.85) ^{b,e}	207.85 (± 36.76) ^{c,f}	209.10 (± 59.36) ^{d,g}	a,b,c,d,e,f $p = 0.001$
20	112.25 (± 24.52) ^{a,b}	204.65 (± 46.68) ^{a,c,d}	166.65 (± 56.31) ^b	142.65 (± 44.37) ^c	147.25 (± 32.28) ^d	a,b,c,d $p = 0.001$
30	78.50 (± 17.81) ^{a,b,c}	142.65 (± 33.64) ^{a,d,e,f}	122.30 (± 32.90) ^{b,d}	93.00 (± 24.51) ^e	111.60 (± 28.18) ^{c,f}	a,b,c,d,e,f $p = 0.001$
45	47.80 (± 10.76) ^{a,b}	98.75 (± 29.15) ^{a,c,d}	69.55 (± 37.82)	53.60 (± 17.97) ^c	70.20 (± 24.48) ^{b,d}	a,b,c,d $p = 0.001$

countries.^{7,18} This decline has been attributed to the use of fluoride-containing materials such as toothpaste.¹⁹ The developing countries have rather observed an increasing prevalence of caries with changes in the dietary pattern.^{3,4}

In Nigeria the rural areas where the very low prevalence of caries was previously observed²⁰ are now experiencing an increase in prevalence because of easy access to cariogenic food^{1,21} and oral health care is not easily accessible to the average Nigerian.²² Majority of the rural dwellers cannot afford toothpaste whilst chewing sticks are more accessible to them. The need to encourage the use of chewing sticks among these communities during our outreach programmes forms the basis of these series of fluoride research in various chewing sticks in Nigeria.

Various researches have demonstrated that topical fluoride can provide anticaries effects through its effect on dental plaque.^{23,24} Other studies have demonstrated that frequent application and retention of topical fluoride in plaque influences microflora within the mouth creating a less cariogenic environment.^{25,26} Some other anticaries properties of topical fluoride include its effect on extracellular polysaccharides formation as well as its effect on bacterial colonization.²⁵ Topical fluoride when used also inhibit the acid-producing ability of dental plaque²⁵ and enhances the remineralization of demineralized enamel¹¹ thereby preventing the dental caries formation.

Brushing time and dentifrice quantity has been demonstrated to be a very important determinant of both oral fluoride retention and consequent enamel remineralization.²⁷ A longer brushing time increases the amount of fluoride retained in the mouth and also increases the fluoride concentration in the mouth 2 hours after brushing.²⁷ Since longer brushing time increases oral fluoride retention this is important and advantageous for chewing stick users, who seem to use the chewing stick for a longer time than toothpaste and brush users.

The population used in studies involving salivary fluoride analysis varies widely and includes ages as low as 4 years to 55 years.^{28,29} A reason attributed to the age variation was that most researchers use participants that are conveniently selected, such as co-staff, dental students and primary or secondary school students. In this present study, we selected school students whose ages ranged between 14–17 years because these age groups are adolescence and are known to be more predisposed to the risk factors of caries. The prevalence of caries is also quite high in this group. The age of participants in this study were similar to those used in a study in Sweden where salivary fluoride was assessed after use of fluoride impregnated chewing sticks and different fluoridated home-care products.^{30,31}

The twenty participants in this present study rinsed their mouth with 10 mL of deionized water for 10 seconds after use of tooth cleaning aids. This contrasts with some other studies where participants rinsed with 10 mL of tap water for 10 seconds.^{32,33} The rationale for not using tap water was to avoid the introduction of fluoride into the mouth from external sources. Ten milliliters of deionized water was used to rinse for 10 seconds to minimize salivary fluoride loss by rinsing. Previous studies have reported that excessive water rinsing post-brushing had a negative impact on salivary fluoride retention.^{29,34}

The retention of fluoride in saliva over time and its clearance is strongly related to individual salivary flow rate.^{35,36} Individuals with normal salivary flow rate have long-lasting fluoride in saliva when compared to people with high salivary flow rate.^{35,36} In this present study, our participants had stimulated salivary flow rate ranging between 0.80 and 2.0 mL/min which fell within the normal range of 1–2 mL/min. It is important that the study was undertaken among participants with normal salivary flow rate to determine fluoride clearance under the normal situation and to standardize results for appropriate comparison with similar studies.

The mean baseline salivary fluoride concentration in this study was 25.95 ppm, this was higher than 0.43 ppm and 7.4 ppm reported for 12–52-year-old Americans and 12-year-old Indians by Zero et al. and Bhargava et al. respectively.^{35,37} The mean baseline salivary fluoride concentration in this present study is high probably because the study area is known to have high fluoride content in the surface water.³⁸ This is comparable to findings obtained by Bhargava et al. and Egbinola et al. in studies carried out in an area with high natural fluoride water.^{37,38} The reason that could be attributed to the higher baseline salivary fluoride concentration may be due to regular intake of water and food prepared from the natural water with high fluoride content.

Non-herbal fluoride toothpaste was selected for the study because it did not contain any herbal ingredient that may mimic results obtained after the use of different chewing sticks. Furthermore, this toothpaste was to serve as an ideal reference for comparing fluoride retention in saliva.

Very few studies had assessed and compared salivary fluoride concentration after use of chewing sticks impregnated with fluoride,^{30,31} but this is the first time natural fluoride-containing chewing sticks were used.

These chewing sticks were selected because they contained the highest fluoride concentration as documented by an earlier Nigerian study which analyzed the fluoride content of selected commonly used chewing sticks.¹² In a study on chewing sticks impregnated with fluoride concentrations between

450 ppm and 1350 ppm, the mean salivary fluoride concentration after use was comparable to that obtained after use of toothpaste containing 1440 ppm of fluoride.³⁰ This can be inferred that natural fluoride-containing chewing sticks could have the same anticaries effect as fluoridated toothpaste.

At sixty minutes the mean salivary fluoride concentrations of the chewing sticks and the non-herbal fluoride toothpaste were above mean baseline salivary fluoride concentration. This is in agreement with previous studies where fluoride was measured from saliva after use of fluoride impregnated chewing sticks and different fluoride-containing products.^{30,32,33}

The observed differences of fluoride retention in saliva immediately after use of chewing sticks and non-herbal fluoride toothpaste over time could be of clinical importance for the prevention and arrest of incipient caries. Since fluoride in saliva inhibits the demineralization of enamel, enhances the remineralization of demineralized enamel and also inhibits production of acid from cariogenic bacteria. The retention of fluoride in saliva after a single application of fluoridated material has been shown to be beneficial for optimum anticaries protection.¹¹

The chewing stick *Zanthoxylum zanthoxyloides* had the highest mean salivary fluoride retention at all timed interval compared to other chewing sticks and the non-herbal fluoride toothpaste. A previous study had reported that *Zanthoxylum zanthoxyloides* had the highest fluoride content among other chewing sticks after fluoride analysis.¹² This may have accounted for the high salivary fluoride concentration after the use of this chewing stick. This salivary fluoride concentration observed over time after the use of *Zanthoxylum zanthoxyloides*, was similar to that seen in a study in which fluoride impregnated chewing sticks had higher salivary fluoride concentration compared to toothpaste with a fluoride concentration of 1450 ppm.³⁹

The non-herbal fluoride toothpaste had the fourth highest salivary fluoride retention at zero minutes but ended up in the second position at sixty minutes. We currently cannot explain this variation in salivary fluoride retention with time but presume it may be related to changing flow rates; further research is needed to explain this phenomenon.

CONCLUSION

Previous studies have determined salivary fluoride retention at timed intervals using chewing sticks impregnated with fluoride, but this is the first time a chewing stick containing fluoride naturally was used. The chewing stick *Zanthoxylum zanthoxyloides* had the highest salivary fluoride concentration after one hour.

CLINICAL SIGNIFICANCE

The use of chewing sticks can be a cost-effective and efficient means of caries prevention if used properly at regular interval. Chewing sticks can be recommended as a cleaning aid during oral health community programmes and for low-income earners for the prevention of caries in rural areas in developing countries.

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