Effects of fluoridated tooth paste on medically erosive enamel in bonded primary teeth during maxillary arch expansion in cleft palate patient: An in vitro study

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Abstract:
AIMS: This study searched the effects of fluoridated toothpaste on the enamel surface of deciduous molars eroded by different pediatric liquid medicaments which were encircled by orthodontic bands by gravimetric analysis method.

MATERIALS AND METHODS: A suitable orthodontic band was used to band each of the 110 non‑carious exfoliated deciduous molars, and stored in artificial saliva. These teeth were divided randomly into fluoridated and non‑fluoridated groups (50 teeth each), and 10 teeth were used as negative controls. The enamel surface of the fluoridated group was swabbed with fluoridated toothpaste for 5 minutes twice daily. Each group was subdivided into five clusters of 10 teeth each according to the type of medication used which included paracetamol, Adol, amoxicillin, Medazole (ME), and Viton (VI) according to a specific immersion cycle. The erosion induced in the enamel for all the teeth was evaluated by gravimetric analysis for different intervals.

RESULTS: The results showed that the weight loss increased with increasing exposure to liquid medication for all groups, but this increase was smaller in the fluoridated group. The highest mean weight loss after 28 days was in VI, and the lowest was in ME (0.145 [0.005] mg; 0.08 [0.008] mg), respectively.

CONCLUSION: The fluoridated toothpaste significantly reduced medically eroded enamel by oral medical syrups. The gravimetric method is valid for the detection of erosion on banded deciduous molars.

Keywords:
Deciduous teeth erosion, oral liquid medications, orthodontic band

Introduction
Children born with a cleft palate have transverse maxillary constrictions.[1] Furthermore, about 8% of the newborn normal children around the globe also present for this transverse maxillary narrowing[2] that is expressed by dental glossary as uni‑ or bi‑lateral posterior crossbite. A palatal expander (PE) could be the only appropriate method to treat these transverse deficiencies.[3] Some clinicians suggest that PE use is mandatory in cleft palate children and the early correction of the transverse posterior crossbite is essential in young children.[4] This is specifically to relocate the follicles of the permanent teeth to a more satisfactory position and to increase the potential for re-establishment of normal occlusal relations in the molars.[4,5]
The anchorage onto the deciduous molars using PE seems to be the appropriate option for early treatment. This could be due to the delay in the eruption of the upper first permanent molars. During early maxillary arch expansion, the deciduous second molars and the deciduous canines are replacing the permanent molars for the PE stability in patients’ mouth. However, the effectiveness of this modification on the PE has been reported by some authors. The replacing of the first permanent molars has some negative side effects during the orthodontic treatment. So, early management using a Haas expander, which is anchored by the deciduous molars and canines, could be acceptable for transverse crossbite resolution as well as for the upper anterior teeth crowding enhancement as it has a simple design and treatment administration.

Enamel erosion has been documented as a problem affecting all age groups. Particularly, enamel wearing that is produced by diet as well as long-term administration of low pH oral medication at an early stage of life. However, the active components of such oral medicaments are essential for the enhancement and/or upkeep of health. At the same time, some of the inactive ingredients have dangerous effects like teeth erosion and caries. Scan electron microscope and surface hardness were the only methods used to evaluate the erosion on the tooth surface.

Since the erosive potential of sugared and acidic oral syrups that are recommended daily to children, especially for those wearing Haas expander or having space maintainer, are improved. Thus, the presence of the molar bands could increase the interjection possibility of retaining the medicine substrate over the deciduous molar for longer time than in normal situations. As a result, the incidence of dental erosion could be higher and the possibility of deciduous molar loss will increase. Finally, the transverse maxillary deficiency and space loss problems will be highly complicated.

**Aims**
The aims of this ex vivo study were to assess the effects of fluoridated toothpaste on medically erosive enamel and the validity of the gravimetric method for the evaluation of the potential hard tissue erosion of different pediatric liquid medicaments induced on the deciduous molars encircled by orthodontic bands.

**Materials and Methods**
The protocol of this study was approved by the Research Ethics Committee of the College of Dentistry / University of Mosul; approval no. 45/1548 in 15/08/2018.

A pilot study was carried out with five samples per group. A sample size assessment and power analysis of its results identified that a sample size of 10 teeth per group would be satisfactory for 85% power with type II error analysis.

In this research, five different kinds of oral syrups recommended for daily use by pediatric physicians were used. These syrups included two analgesics Paracetamol (PA) (TIS Farmaceutic, Bucharest, Romania) and Adol (AD) (Atabay Ilac, Istanbul, Turkey); one antibiotic (Amoxicillin (AM) [Khalid Pharmacy, Lahore, Pakistan]); one antifungal (Medazole (ME) [Pioneer Pharma, New Cairo, Egypt]); and one iron supplement (Viton (VI) [Silco Pharma Ltd., Sylhet, Bangladesh]). Table 1 shows the composition and the pH of these syrups. The pH values of these selected medicines at room temperature were measured using a pH meter (Digital pH Meter, Philips, Japan).

**Sample collection**
The exfoliated non-carious deciduous molars were collected from the pediatric department of the dental center at the College of Dentistry / University of Mosul. Filled or erosive teeth were excluded from this study. A total of 110 teeth were fit for the inclusion criteria. All the teeth (110) were hand-scaled, cleaned, and well-examined by a dentist for structural distortion that could hinder the outcome. Each tooth was banded with a suitable stainless steel orthodontic band size (Dentaurum, Ispringen, Germany). Then, these bands were cemented by Fleck’s self-cure Snow White zinc phosphate cement (Keystone Industries, Singen, Germany). All the cement accessories were removed before the teeth were stored in artificial saliva at 37°C for subsequent processing.

**Sample grouping**
The 110 final included teeth were divided into two experimental groups randomly as the first 50 teeth to be fluoridated (FG) and the last 50 teeth to be non-fluoridated (NG). While the remaining 10 teeth were used as a negative control group. The samples of each group were remarked by specific colors to ensure the blindness of the operators. Two operators cooperated to complete the practical portion of this research. To ensure the blindness, the first operator marked the groups and the second operator performed the testing procedures. The negative control group is that group which is not exposed to any medications and is just stored in artificial saliva for the same intervals as the other group [Figure 1]. In the NG group, no material was added to the teeth surfaces whereas, in the FG, the enamel surface of each tooth was swabbed with a thin layer of fluoridated toothpaste (Maclean training toothpaste, Glaxo Smith Kline, Brentford, UK) using a fine brush for 5 min. This procedure was performed twice daily to simulate normal oral hygiene instructions. A thin smear layer of the toothpaste was swabbed by the brush and applied.
over the occlusal surface of the teeth as the whole surface was covered by the toothpaste. Then, all the samples were re-immersed in artificial saliva. Each experimental group was subdivided into 5 clusters of 10 teeth each, according to the five types of medication used again, the selection was done randomly. Each of these clusters was exposed to the tested medications according to a specific immersion cycle (see further). Between each immersion cycle, the teeth were re-stored in artificial saliva at 37°C for 24 h in separated incubators (for each cluster of teeth). The artificial saliva was prepared according to Taqa et al.\cite{16} and its components are listed in Table 2.

**Immersion cycles**

The protocol of sample immersion was derived from the previously published method subscribed by Kulkarni.

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**Table 1: The composition and the mean pH of the medications used in this study**

| The Medication       | Composition                                                                 | pH   |
|----------------------|-----------------------------------------------------------------------------|------|
| Paracetamol (PA)     | Paracetamol 120 mg/5 mL                                                     | 5.11 |
| Adol (AD)            | Paracetamol 100 mg/1 mL                                                     | 5.12 |
| Amoxicillin (AM)     | Amoxicillin as trihydrate USP 250 mg                                        | 5.08 |
| Medazole (ME)        | Metronidazole as benzoyl Bp 200 mg                                         | 6.19 |
| Viton (VI)           | Akkal kara (Anacyclus pyrethrum 15.00 mg)                                  | 4.94 |
|                      | Ashwagandha (Withania somnifera) 30.00 mg                                  |      |
|                      | Dalchini (Cinnamomum zeylanicum) 3.00 mg                                   |      |
|                      | Elaychi (Elettaria cardamomum) Taj (Cinnamomum cassia) 3.00 mg             |      |
|                      | Naugkeshar (Mesua ferrea) 3.00 mg                                          |      |
|                      | Gohgaru (Tribus terrestis) Ext 5.00 mg                                    |      |
|                      | Jaylal (Myristica fragrans) 10.00 mg                                       |      |
|                      | Kaucha Beej (Mucuna prurienis) Ext 5.00 mg                                 |      |
|                      | Majith (Rubia cordifolia) 10.00 mg                                         |      |
|                      | Raisan (Pluchea lanceolata) 10.00 mg                                       |      |
|                      | Satavari (Asparagus racemosus) 25.00 mg                                    |      |
|                      | Shilajeet Shuddha (Asphaltum panjabinum) Ext 7.50 mg                      |      |
|                      | Amla (Phyllanthus emblica) 5.00 mg                                         |      |
|                      | Baheda (Terminalia bellirica) 5.00 mg                                       |      |
|                      | Harde (Terminalia chebula) 5.00 mg                                         |      |
|                      | Sunth (Zingiber officinale) 5.00 mg                                        |      |
|                      | Kali Mirch (Piper nigrum) 5.00 mg                                          |      |
|                      | Pippali (Piper longum) 5.00 mg                                             |      |
|                      | Vidhara (Argyria speciosa) Ext 5.00 mg                                     |      |
|                      | Vidarikand (Pueraria tuberosa) 20.00 mg                                    |      |
|                      | Flavored sugar syrup base Q. S.                                           |      |
|                      | Color: Carmoisine and Sunset Yellow FCF                                    |      |

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**Figure 1: Schematic study design and sample grouping**
Table 2: Composition of artificial saliva per one liter of water (Taqa et al., 2019)

| Component                        | Weight | pH  |
|----------------------------------|--------|-----|
| Artificial Saliva                |        |     |
| CaCl$_2$                         | (15 mg**) |     |
| MgCl$_2$                         | (5 mg**) | 7   |
| KCI                              | (0.1 g*) |     |
| KSCN                             | (10 mg**) |     |
| Na$_2$HPO$_4$                    | (40 mg**) |     |
| Sodium carboxymethylcellulose    | (1.0 g*) |     |
| Methylparaben                    | (0.1 g*) |     |

*g gram, **mg milligram

In this method, the occlusal surface of the molars was adapted to simulate the daily consumptions using a stainless-steel clamp to ensure that the inferior surface of the crown was not soaked in the medication. Each tooth was dipped for 1 min in bottles containing 5 mL of the syrup under investigation. This procedure was repeated three times per day with 6 h interim between the immersion series. Then, the teeth were rinsed with deionized water and were well-dried for erosive evaluation (see further). After each immersion cycle, the teeth were maintained in 10 mL of the previously prepared saliva at 37°C until the subsequent immersion phase. The syrups were renewed before each immersion series.\(^{[17]}\)

**Teeth erosion evaluation**

The erosion of the deciduous teeth was evaluated using an updated method designated by Von Fraunhofer and Rogers.\(^{[18]}\) This method depends on the assessment of the weight loss of the tooth before and after exposure to the erosive factor. So, all the teeth were dried using a blotting paper at room temperature for 1 h and were then weighted using a calibrated accuracy balance (Sartorius BP61S, Göttingen, Germany)—the metering precision was 0.1 mg. The above procedures were repeated after 7, 14, 21, and 28-day intermissions after the constant and regular repetition of the daily immersion series. The negative control group (10 teeth) were stored in the artificial saliva throughout the experiment period (28 days) with a regular daily refreshment of artificial saliva for all clusters. Figure 1 shows the study design and sample grouping.

**Composition of toothpaste**

The toothpaste used in this study was composed of sodium mono-fluoro-phosphate (500 ppm fluoride), sorbitol, aqua, hydrated silica, glycerin, titanium dioxide, sodium lauryl sulfate, xanthan gum, di sodium phosphate, aroma, sodium saccharin, garageenan, calcium glycerophosphate, and limonene CI77492, CI77491 (Maclean Training Toothpaste, Glaxo Smith Kline, Brentford, UK).

**Statistical evaluations**

The outcome data were typed into an Excel sheet. The statistical exploration was done using the statistical package for the Social Sciences software (version 19). The descriptive analysis (mean, standard deviation) was used to find the weight loss for all teeth and at different intervals. One-way ANOVA was also used to find the significant differences between the tested groups. P value was adjusted to be \(P < 0.05\).

**Results**

The physiochemical properties and the pH of each pediatric medicinal syrup are presented in Table 1. All the studied syrups were acidic on opening, although the pH of ME was higher than the pH of the remaining syrups (6.19). The pH of VI was lower than the pH of the remaining syrups (4.94).

Among all liquid medications used in this study, the mean of weight loss after 28 days was the maximum in VI (0.145 SD [0.005 mg]) while it was the least in ME (0.080 SD [0.008 mg]). On the other hand, NG had a higher teeth weight loss than FG under the same type of liquid medication used [Table 3].

Figure 2 shows that there was considerable weight loss of tooth in each medication, especially after 28 days. The graph also shows that the mean of the total teeth weight loss was in the VI, when comparing it with the negative control group, the tooth weight loss was higher in VI after 7, 14, 21, and 28 days. However, other medications also showed higher means for total tooth weight loss when compared with the negative control group. The result also showed that the weight loss increased with the increasing frequency of exposure to liquid medication for all groups.

**Discussion**

In this ex vivo study, the amount of erosion induced in the deciduous teeth was expressed as weight loss. This gravimetric analysis method has been reported in the literatures and accepted internationally as it is simple, feasible, and meaningful.\(^{[19‑21]}\)

Recently, teeth erosion has been significantly documented as the basis of tooth structure loss in children and adolescents\(^{[22]}\) especially for banded deciduous molars used in expansions of the constricted maxilla or for space maintainer purposes. The bands as well as all the components of the expander are considered as rough, irregular surfaces that can retain the medications and food remnants and increase the time of exposure of these erosive materials on the teeth enamel. However, in this study, we try to simulate the oral environments by banding the teeth with orthodontic bands before exposure to oral medicine.

The current research indicates that the investigated syrups could erode the primary tooth enamel after the
Qasim, et al.: Gravimetric analysis of the effects of fluoridated toothpaste on medically erosive enamel of the primary teeth encircled by orthodontic band in cleft palate patients: An in vitro study

Research on primary teeth substrates is of scientific relevance as it has been observed that there is a structural and morphological difference between the deciduous and permanent teeth. The dissimilarities in the chemical conformation, rate of development, and ultra-structure have been inspected between the pellicle on the primary and permanent teeth. The primary enamel is more susceptible to enamel erosion with 50% more mineral loss and 30% more lesion depth.\[23\] The primary enamel is less mineralized and the density of its outermost layer is lesser when compared to the permanent teeth.\[23\] As a result, the loss of the anchor deciduous molars may lead to vital failure of the expansion in the palatal constricted maxilla.

We followed the previously authorized methods for erosive evaluation and immersion cycle of the oral medications used.\[17‑19\]

In the present study, the five investigated syrups have acidic components, which decreased the pH of the oral cavity and saliva as shown in Table 1. However, the present study showed that the pH of ME was higher than the pH of the remaining syrups (6.19). While, the pH of VI showed the lowest value among all the remaining syrups (4.94), and the pH of all the pediatric syrups used in the present study were lower than the critical pH of demineralization which reported to be 5.5 except for the ME syrup as the probability of enamel erosion increases with decreasing pH.\[17\]

During syrup production, the acids are usually added to the preparation as a buffering tool to preserve its chemical constancy in addition to regulating the tonicity and certifying its physiological compatibility. Another reason for acid addition is to enhance flavor and increase palatability for children.\[12\] However, citric acid, despite its weak acidity, has recently been reported to have the ability to be erosive to the enamel surfaces of the tooth

Table 3: Comparison of mean tooth weight (mg) loss after immersion into each medication and in four intervals of 7th, 14th, 21st and 28th days

| Type of medication | Tooth loss 7th day Mean (SD) | P* | Tooth loss 14th day Mean (SD) | P* | Tooth loss 21st day Mean (SD) | P* | Tooth loss 28th day Mean (SD) | P* |
|--------------------|-----------------------------|----|-----------------------------|----|-----------------------------|----|-----------------------------|----|
| Viton              | 0.025 (0.005)               | 0.003 | 0.065 (0.005)               | 0.0 | 0.105 (0.005)               | 0.0 | 0.145 (0.005)               | 0.0 |
| Viton with Fluoride| 0.008 (0.0)                 | 0.0 | 0.025 (0.005)               | 0.003 | 0.055 (0.005)               | 0.0 | 0.080 (0.008)               | 0.0 |
| Amoxicillin        | 0.015 (0.0)                 | 0.0 | 0.050 (0.008)               | 0.001 | 0.080 (0.008)               | 0.0 | 0.120 (0.008)               | 0.0 |
| Amoxicillin with Fluoride | 0.005 (0.0) | 0.001 | 0.015 (0.0)               | 0.0 | 0.040 (0.008)               | 0.002 | 0.070 (0.008)               | 0.0 |
| Paracetamol        | 0.010 (0.0)                 | 0.0 | 0.040 (0.008)               | 0.002 | 0.070 (0.008)               | 0.0 | 0.110 (0.008)               | 0.0 |
| Paracetamol with Fluoride | 0.005 (0.0) | 0.001 | 0.015 (0.0)               | 0.0 | 0.035 (0.0)               | 0.0 | 0.065 (0.0)               | 0.0 |
| Adol               | 0.008 (0.0)                 | 0.005 | 0.030 (0.0)               | 0.0 | 0.060 (0.0)               | 0.0 | 0.100 (0.0)               | 0.0 |
| Adol with Fluoride | 0.005 (0.0)                 | 0.001 | 0.013 (0.0)               | 0.0 | 0.035 (0.0)               | 0.0 | 0.060 (0.008)               | 0.001 |
| Medazole           | 0.008 (0.0)                 | 0.0 | 0.015 (0.0)               | 0.0 | 0.050 (0.0)               | 0.001 | 0.080 (0.008)               | 0.0 |
| Medazole with Fluoride | 0.004 (0.0) | 0.0 | 0.010 (0.0)               | 0.0 | 0.050 (0.00)               | 0.001 | 0.060 (0.0)               | 0.0 |
| Negative Control   | 0.001 (0.0)                 | 0.0 | 0.002 (0.0)               | 0.0 | 0.004 (0.0)               | 0.0 | 0.005 (0.0)               | 0.0 |

*P<0.05

Figure 2: Schematic tooth loss by weight after immersion interval on 7th, 14th, 21st, and 28th days for fluoridated and non-fluoridated groups and for all used medications.
due to the chelating capability of the calcium in the hydroxyapatites.\textsuperscript{11}

Our results showed an erosive in the enamel of the banded teeth, which was expressed in this study as a weight loss in all the tested groups on the 7th day as compared to the negative control values. The VI syrups showed the maximum means of weight loss and it was statistically significant. The reason for more weight loss on the 7th day as compared to negative control group\textsuperscript{P} values could be due to syrups exposure. On the other hand, this difference observed in different groups could be related to the variations in the syrups’ pH as well as the used acids, the buffers added, and finally could be due to the compositions of the oral liquid medicine.\textsuperscript{24} Supposing that the enamel for all the teeth has the same mineralization components.

On the other hand, the recent study indicated that the mean of the total tooth weight loss on the 28th day was the highest in VI, 0.145 (SD 0.005) mg, and least in ME, 0.080 (SD 0.008) mg. This may be because ME has a higher pH than the remaining syrups (6.19).

The 4-week (28 days) investigating period was selected to study the effects of long-term administration of the medicine. Particularly, for young children with PE. It seems that extended treatments could induce high tooth structure loss, and that could be greater than those marked in this investigation. However, a similar protocol was followed by Amaechi et al.\textsuperscript{25} but they used orange juice to evaluate the erosive potential of bovine teeth after a 1 h immersion cycle. Nevertheless, the oral liquid medicines investigated were nominated because they are routinely prescribed for the treatment of childhood illnesses.

Similar to the \textit{in vitro} study by Scatena \textit{et al.}\textsuperscript{26} who assessed the erosive potential of pediatric liquid medicines in primary tooth enamel, the syrup immersion cycles were done for 1 min, three times per day for 4 weeks. The tooth surface microhardness was competed at 1, 2, 3, and 4 weeks, respectively. Additionally, the scanning electron microscope was used to show the tooth surface on the 28th day, which clearly exhibited structural loss.\textsuperscript{26}

The erosive potential of liquid oral medications might be related to the regularity and interval of acid contact. It is also related to the total volume of the syrups ingested. The increase of the exposure of the syrup to the teeth surfaces will increase the potential of teeth erosion. Despite the properties of these oral syrups, the indiscriminate consumption of child syrups, particularly by young children, could increase dental erosion and tooth loss. Even more, the administration of liquid oral syrups before bedtime could worsen the condition, particularly, if it is not followed by good oral hygiene.\textsuperscript{27}

The result of the current study also showed that increase of weight loss with time was smaller in all the groups which used fluoridated toothpaste before getting exposed to medication as a preventive protocol to reduce the amount of teeth erosion induced by the oral medication that is daily prescribed by physicians to treat different sicknesses. This may be attributed to the formation of precipitated calcium fluoride (CaF\textsubscript{2}) that poses as a reservoir of fluoride that releases fluoride ions gradually upon acid dose,\textsuperscript{28} and this also, highlights the importance of oral health care with fluoride supplements especially for young children with cleft palate, under fixed orthodontic therapy by PE.

Therefore, the use of child’s syrup necessitates concentrated oral hygiene measures with fluoridated toothpaste.\textsuperscript{29} Also, it is well-thought-out that the pharmacological companies should advertise the possibility of tooth erosion on the drug leaflet.

\textbf{Conclusion}

The gravimetric method is valid for the detection of erosive enamel induced by liquid medications on banded deciduous molars, and the fluoridated toothpaste has a significant reduction of such erosion potential. Most oral medications used for children were ignorant concerning the sweetening agents and acidity, which cause aggressive effects on the teeth enamel. Also, high care should be considered after prescribing liquid medications, especially for young children with constricted maxilla and under fixed orthodontic treatment. Hence, brushing with fluoridated toothpaste should instructed to enhance the oral health related to the quality of life in such children.

\textbf{Limitations of the study}

The limitations of this \textit{ex vivo} study could include the following: The teeth were immersed for 1 min in each medication. However, \textit{in vitro} study might not reproduce the results of an \textit{in vivo} one. Besides, the effect of oral factors such as saliva and dental plaque on the enamel re-mineralization and mineral loss from tooth surface could not be considered.

\textbf{Clinical implication of the study and recommendations}

This study shows the importance of oral hygiene instructions for young children. Especially, for those having Haas expander or space maintainer, as the deciduous teeth are encircled with molar bands. The use of fluoridated toothpaste before oral consumption of liquid medicine highly reduces the erosive potential of the deciduous teeth and increase their survival time.
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Conflicts of interest
There are no conflicts of interest.

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