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RESEARCH ARTICLE

“EFFECT OF APPLICATION OF REMINERALIZATION AGENTS ON MICROHARDNESS & SURFACE ROUGHNESS OF THE ENAMEL SURFACE AFTER INTERPROXIMAL STRIPPING – IN VIVO STUDY”.

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Abstract

Background and objectives: Interproximal enamel stripping is in practice clinically in orthodontics to obtain more space to align crowded teeth. Any product that might prevent mineral loss and promote remineralization is worth investigating. Therefore, the aim of this study was to investigate and to compare the effects of a commercial paste of xylitol and fluoride application after interproximal stripping of enamel surfaces.

Method: sixteen patients undergoing orthodontic treatment participated in this study. For each patient, the extraction of 4 first premolars was part of orthodontic treatment plan. The patients were segregated into 4 groups of 4 patients. In group 1, no stripping was performed, and the teeth were removed immediately. In group 2, the stripped teeth were extracted after exposure to oral conditions for 3 months. In groups 3 and 4, xylitol toothpaste or fluoride was applied to the stripped surfaces for 3 months, respectively, before the teeth were extracted. Microhardness values were evaluated with anova one-way analysis of variance and tukey HSD tests.

Result: stripping decreased the microhardness of the enamel. However, the mean values of enamel microhardness increased after the xylitol and fluoride gel application. Fluoride showed marginally more amount of remineralization than xylitol which is statistically significant. (p<0.001)

Conclusion: Based on the findings of this in vivo study, it can be

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concluded that the saliva and remineralizing agents (fluoride and xylitol) increased the microhardness values of stripped enamel surfaces.

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Introduction:-

Interproximal reduction is a clinical method involving reduction, anatomic recontouring and protection of proximal enamel surfaces of permanent teeth. (Peck and Peck 1972). Several studies have investigated the harmful effects of proximal stripping due to loss of the protective superficial enamel layer. [Sheridan et al,1989; Zachrisson et al , 2007, 2011; Arman et al, 2006]

Few studies claim that any injury to sound proximal enamel surface by stripping can lead to caries and periodontal problem. [Arman et al,2006; Radlanski et al,1988; Mikulewicz et al,2007] .However to prevent the undesirable side effects of interproximal stripping, it is advised to use polishing discs and some agents (fluoride products and sealants to produce a smoother enamel surface and enhancing remineralization.[Sheridan et al,1989; Danesh et al,2007; Mikulewicz et al,2007; Joseph et al,1992; Rossouw et al,2003]

In relation to this, any product that might prevent mineral loss and promote remineralization is a topic for further research. Xylitol is a non-toxic sugar alcohol sweetener which is resistant to fermentation by the streptococcus mutans [Elbrahim et al, 2010] . Xylitol forms complexes with calcium ions & prevents precipitation of calcium phosphate, hence facilitate transport of calcium and phosphate ions for remineralization. [Steckse n-Blicks et al, 2004]

Fluoride adheres to enamel resulting in a substantial and prolonged increase in the level of fluoride in the oral environment. In addition to its remineralizing action it also acts as a physical barrier that protects the enamel against acid attack [Reynolds et al,1998]. The use of fluoride to prevent white spots during orthodontic treatment has been widely investigated compared to their effects following interproximal reduction procedures. [Heshmat et al,2016; Chow et al,2001; Chow et al,1990]

So, the aim of this study was to investigate and to compare the effects of a commercial paste of xylitol and a fluoride gel application after interproximal stripping of enamel surfaces. The research hypotheses is that the remineralizing agent application (1) will not alter the roughness and (2) will not increase the microhardness of the stripped enamel surfaces.

Aim:-

To investigate and to compare the remineralizing effect on the microhardness & surface roughness of enamel by application of a commercial paste of xylitol and a fluoride gel after interproximal stripping of enamel surfaces

Objectives:-

1. To investigate after interproximal stripping of enamel surfaces the remineralization effect by application of a commercial paste of xylitol on microhardness & surface roughness of enamel
2. To investigate after interproximal stripping of enamel surfaces the remineralization effect by application of a fluoride gel on microhardness & surface roughness of enamel
3. To compare the remineralizing effects by application of a commercial paste of xylitol and a fluoride gel on microhardness & surface roughness of enamel after interproximal stripping.

Materials & Methodology:-

Sixteen patients in the age group of 13 – 23 with skeletal Class I malocclusion participated in the study. For each of these patients, the extraction of 4 first premolars was part of the treatment plan. Before starting the study, a thorough information about the study design was given and consent form was signed from all adult patients and the parents of those under 18 years of age. This study protocol was approved by research & ethical committee at Manubhai Patel dental college, hospital and ORI, Vadodara(MPDC_117/ORTHO_23/17). All patients were given oral hygiene instructions and monitored.

Inclusion criteria:-

The permanent maxillary & mandibular premolars to be extracted for therapeutic purposes were selected.

Exclusion criteria:-

Teeth with visible and detectable caries, hypoplastic lesions, stains, white spot lesions.

The interproximal enamel stripping was performed on both mesial and distal surfaces of the premolars in the stripping groups. Before starting stripping procedure, in all subjects elastic separators (Fig 1) were placed on the mesial and distal contacts of the premolars for 3 days as a precautionary measure.

The teeth were then stripped with a metal strip with abrasive particles on one side (Strauss Abrasive Strips Metal S/S 4X150 mm). A new abrasive strip per patient was used. (Fig 2 A,B)

The patients were divided into 4 groups of 4 patients (16 premolars, 32 surfaces in each group) according to the following procedures.

Group A: sound enamel (control)

Group B: stripped enamel surface exposed to saliva

Group C: stripped enamel surface after application of Xylitol toothpaste (Spry toothpaste fluoride free)+ saliva exposure and

Group D: stripped enamel surface after fluoride gel application (1.23% APF gel) + saliva exposure

Group a	Group b	Group c	Group d
No stripping was performed, and the premolars were removed immediately	After proximal stripping of 1st premolars it was exposed to oral conditions for 3 months.	After proximal stripping a Xylitol toothpaste was given to the patient & a demonstration of how to apply was given by clinician at the 1st appointment. Patients were told to treat the stripped surfaces with Xylitol after brushing their teeth according to the instructions given for 3 minutes once a day for 3 months. They were advised not to eat or drink for 30 minutes after application. The teeth were then extracted after 3 months for evaluation	After proximal stripping isolation was done with cotton roll, the stripped surfaces of the teeth were gently dried with air. In accordance with the manufacturer's instructions, the fluoride gel was applied to the stripped surfaces by using a handle and brush tip once a month for 2 months. The gel was then air-dried for 10 to 20 seconds, and the treated surface was protected from salivary contamination for a further 20 to 25 seconds. The patients were told not to wash with water, not to eat or drink anything for 3 hours. The teeth were then extracted after 3 months for evaluation

All extracted teeth (n = 64) were collected and stored in normal saline until needed. After the root portion of the crown was removed, each tooth crown was sectioned into 2 halves in the buccolingual direction using a carbide disc. Thus, 128 enamel slices were obtained. To carry out the microhardness and surface roughness tests, the teeth were embedded in self-curing acrylic resin, leaving the proximal enamel surfaces uncovered. (Fig 4)

Vickers hardness was tested with a Micro Hardness Tester (Vickers Cum Brinell Hardness tester with standard accessories Load range) by applying a square-base pyramidal diamond indenter on the stripped surface of the tooth sections under a load of 50 g for 10 seconds (as shown in Figure 5 & 6). The samples were stabilized parallel to the base of the hardness measurement device For each specimen, 3 indentations were made, and the average was calculated.

The determined values were averaged to represent the Vickers hardness value of that specimen.

For the SEM evaluation, 4 specimens (1 sample from each group) were prepared to evaluate the enamel surfaces qualitatively. The photomicrographs were taken with a SEM (JEOL JSM-5610LV) with 500-times magnification for visual inspection (Fig 8)

Sample size:-

Minimum 128 (32 per group) observations required for present study to get mean difference of surface roughness by 0.07 with SD of 0.08 at 5% risk and 80% power.

Statistical Analysis:-

1. Data analyses were performed by using following Statistical tests:
2. Descriptive statistics (mean, SD, CI)
3. One way ANOVA test
4. Post Hoc test for multiple comparisons

The data showed normal distributions, and there was homogeneity of variances between the groups. One-way analysis of variance (ANOVA) was performed to inspect the effects of the remineralization agent application on the stripped enamel surface. The Tukey HSD test was used for post hoc comparisons of the groups. The results were evaluated with a 95% confidence interval. The significance level was set at $P < 0.05$.

Results:-

The SEM photomicrographs (500-times magnification) of the intact and stripped enamel surfaces are shown in figure 8

SEM images showed deep furrows were formed on the proximal surface stripped with abrasive proximal strip in group 2 whereas in group 3 & 4 a layer is seen on the surfaces stripped with a hand pulled strip after xylitol & fluoride gel application respectively, which forms an outer protective layer.

The results of **Table 1 & 2** shows a statistically significant difference ($P < 0.001$) between the groups for microhardness.

The results showed that stripping decreased the microhardness of the enamel. However, the mean values of enamel microhardness increased after the Xylitol and fluoride gel application in groups 3 and 4, respectively. The microhardness in xylitol group was 312 with SD of 5.78 whereas in fluoride gel group it was 317.44 with a SD of 5.715

Hence, Fluoride showed marginally more amount of remineralization than xylitol & hence increased the microhardness of tooth post stripping. A graphic representation of the changes in microhardness values is shown in figure 7.

Discussion:-

Interproximal enamel stripping is a common orthodontic procedure to gain space for crowded teeth as well as to correct Bolton tooth-size discrepancies, and to eliminate black triangles between adjacent teeth caused by gingival recession. [Peck & Peck et al,1972; Sheridan et al,1989; Zachrisson et al,2007; Zachrisson et al,2011] Although a direct relationship could not be detected between the stripping procedure and the increased susceptibility to caries and periodontal diseases, some preventive strategies have been recommended after enamel stripping to reduce possible detrimental effects. [Sheridan et al,1989; Mikulewicz,2007; Rossouw et al,2003]

The increased remineralization effects of fluoride and Casein phosphopeptide–amorphous calcium phosphate (CPP–ACP) have been shown in many previous in-vitro studies by qualitative and quantitative analyses, but the results of these studies were limited in assessing the clinical effectiveness because of many factors that are difficult to reproduce, such as salivary flow and contents, temperature changes, oral hygiene, and dietary intake in individual patients. [Sheridan,1989] The aim of this in- vivo study was to evaluate and compare the effects of remineralizing

agents (fluoride gel and xylitol paste), as well as saliva, on stripped enamel surfaces.

The role of saliva, fluoride, and CPP-ACP application on the remineralization process have been described in detail in previous investigations. [Amaechi et al, 2005; Reynolds et al, 1998; Chow et al, 2001] Saliva is a natural source of inorganic ions necessary for remineralization. It provides protective effects with its buffering and remineralizing capacity in addition to neutralizing and clearing the acid. [Amaechi et al, 2005; Heshmat et al, 2016] CPP localizes ACP on the surface of tooth, which then buffers the free calcium and phosphate ions, thereby helping to maintain a saturated & concentrated state with respect to the enamel, thus decreasing demineralization and promoting remineralization. [Reynolds et al, 1998] Fluoride is the most well-known and widely used remineralizing agent. It is generally accepted that the beneficial effects of fluoride arise from its incorporation in the tooth mineral as fluoroapatite or fluoride-enriched hydroxyapatite, both leading to decreased solubility of tooth enamel. [Chow et al, 1990; Chow et al 2001]

Xylitol is a non-toxic sugar alcohol sweetener, and resistant to fermentation by the streptococcal caries-inducing bacteria. Xylitol forms complexes with calcium ions thereby preventing more general calcium phosphate precipitation, facilitate transport of calcium and phosphate ions for remineralization of demineralized enamel.

E Ibrahim-Auerkari, et al [26] investigated in vitro effects of xylitol exposure on the remineralization as reflected in structure, composition and hardness of demineralized enamel. The results showed significantly higher mean enamel hardness after immersion in the xylitol-containing remineralization solution in comparison with the demineralized samples ($p < 0.05$). Giullio et al [18] investigated in vitro the effect of CPP-ACP application on stripped enamel surface morphology with the SEM after exposure to a lactic acid solution. They concluded that topical applications of CPP-ACP could be effective in promoting enamel remineralization after interdental stripping. Paganelli et al [19] assessed in vivo the morphology and composition of the interproximal stripped enamel after exposure to saliva and CPP-ACP with sodium fluoride. Their results showed no difference between the effects of saliva and CPP-ACP with sodium fluoride on stripped enamel surfaces after 30 days of exposure. Sengun found that Xylitol lozenges can significantly reduce the acidity of dental plaque in fixed appliance patients. [28] The Xylitol lozenges helped in neutralizing the acidity of dental plaque after the administration of sucrose in orthodontic patients with fixed appliances. Thaweboon et al [29] conducted a study to determine the remineralization effects of xylitol chewing gum containing funoran and calcium hydrogenphosphate on enamel subsurface lesions in humans & concluded that Chewing xylitol gum containing funoran and calcium hydrogenphosphate has a significant effect on the remineralization of initial caries-like lesions of the teeth.

In a study by Milake et al [30] they morphologically determined the effects of xylitol on the remineralization of artificially demineralized enamel and concluded that xylitol can induce remineralization of deeper layers of demineralized enamel by facilitating Ca^{2+} movement and accessibility.

The present study was performed to evaluate and to compare the effects of a commercial paste of xylitol and a fluoride application after interproximal stripping of enamel surface. The results of this study demonstrated that the highest mean microhardness value was found in the intact enamel group (group 1, no stripping), and the lowest microhardness value of stripped enamel was seen in group 2, where the teeth were removed after stripping enamel surface was exposed to saliva for 3 months In Xylitol (group 3) and fluoride gel (group 4), the mean microhardness values were greater than those of group 2. A statistically significant differences were found between all the groups. These results demonstrated that saliva, fluoride gel, and Xylitol increased the mineral contents and the microhardness of stripped enamel. The changes in groups 3 and 4 were the results of the combined effects of saliva and the remineralizing agent.

Previous studies that evaluated the stripped enamel surfaces with SEM have shown that all stripping methods affect enamel surface morphology, leaving furrows and scratches. In study by Agrawal et al [31] SEM images showed that Tungsten carbide bur produced a finely rough surface which was smoother when compared to the deep uniform furrows formed with hand pulled proximal strip. However an outer uniform layer was formed after fluoride application on the stripped surface which prevents its demineralization from oral acid. So it was concluded that proximal stripping with tungsten carbide bur followed by application of fluoride varnish or bonding agent was

preferable.

According to our results, Group 2 had a relatively rough surface compared to group 3 & 4 in which a layer is seen on the surfaces stripped with a hand pulled strip after xylitol & fluoride gel application respectively, which forms an outer protective layer.

Although the split-mouth study design is frequently used in oral health research, it was not preferred in this n-vivo study because there was a high risk regarding the mixing of the effects of the remineralizing agents, which were soluble in oral conditions. A limitation of this study design was the sample size. Even if the numbers of samples (surfaces) in each group were considered sufficient for statistical analysis, the number of subjects in each group was small. Some factors influencing the remineralization process, such as oral hygiene, saliva properties, and dietary intake, are variable between patients. To minimize the effects of these variations on the study results, further studies are needed with larger groups.

Conclusion:-

Within the limitations of this in-vivo study, the following conclusions may be drawn.

1. Interproximal enamel stripping decreased the microhardness values of enamel surfaces. The saliva and remineralizing agents (fluoride and Xylitol) did increase the microhardness values of stripped enamel surfaces that were decreased by stripping.
2. In terms of the microhardness, there was a statistically significant difference between the effects of Xylitol and fluoride gel applications on stripped enamel, as well as saliva. The microhardness was least in saliva group (288 with a SD of 4.719) The microhardness increased in xylitol group (312 with SD of 5.78) whereas in fluoride gel group it was highest (317.44 with a SD of 5.715).
3. With regard to surface roughness by SEM evaluation, stripped enamel exposed to saliva for 3 months showed a rougher surface compared to control, xylitol & fluoride groups.
4. Fluoride showed marginally more amount of remineralization than xylitol which is statistically significant & hence increased the microhardness of tooth.

Figure 1:-Elastic separators



Figure 2 (A, B):-Strauss Abrasive Strips Metal S/S 4x150 Mm



Figure 3:-Spry Xylitol toothpaste (fluoride free)



Figure 4:-Sample embedded in self cure acrylic



Figure 5:-Vickers Cum Brinell Hardness tester with standard accessories Load range

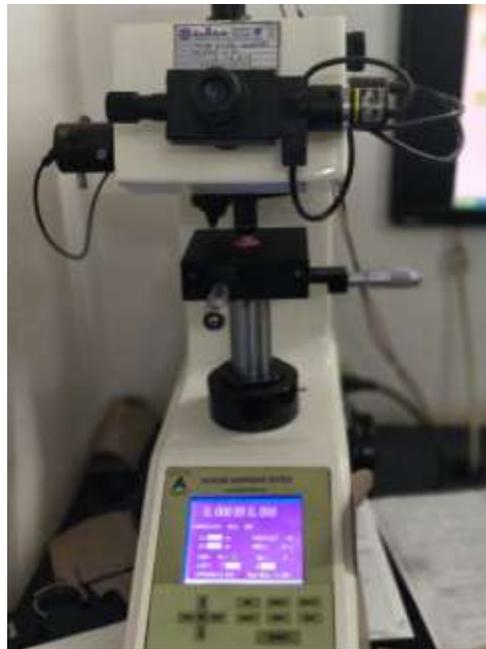


Figure 6:-Samples were stabilized parallel to the base of the hardness measurement device

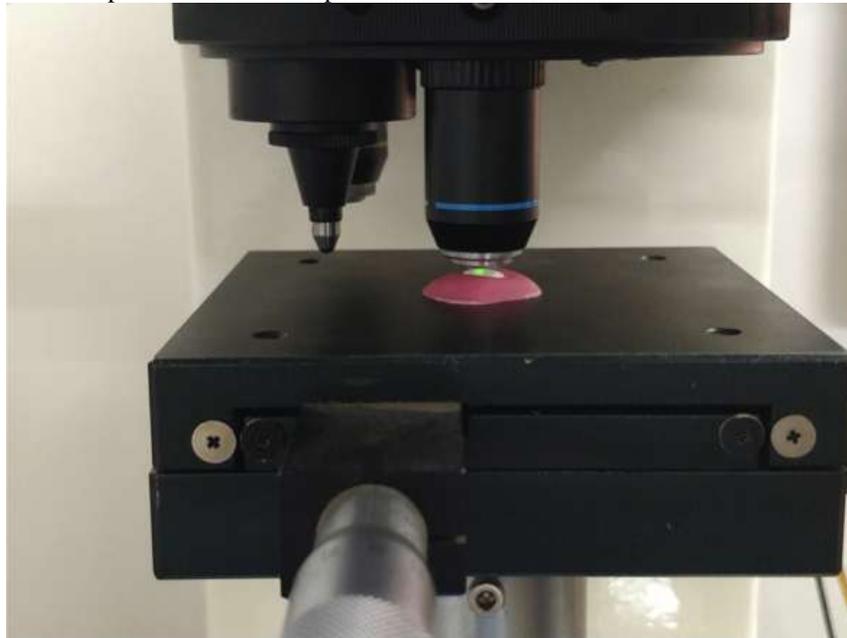


Figure 7:-Graphic representation of the changes in microhardness values

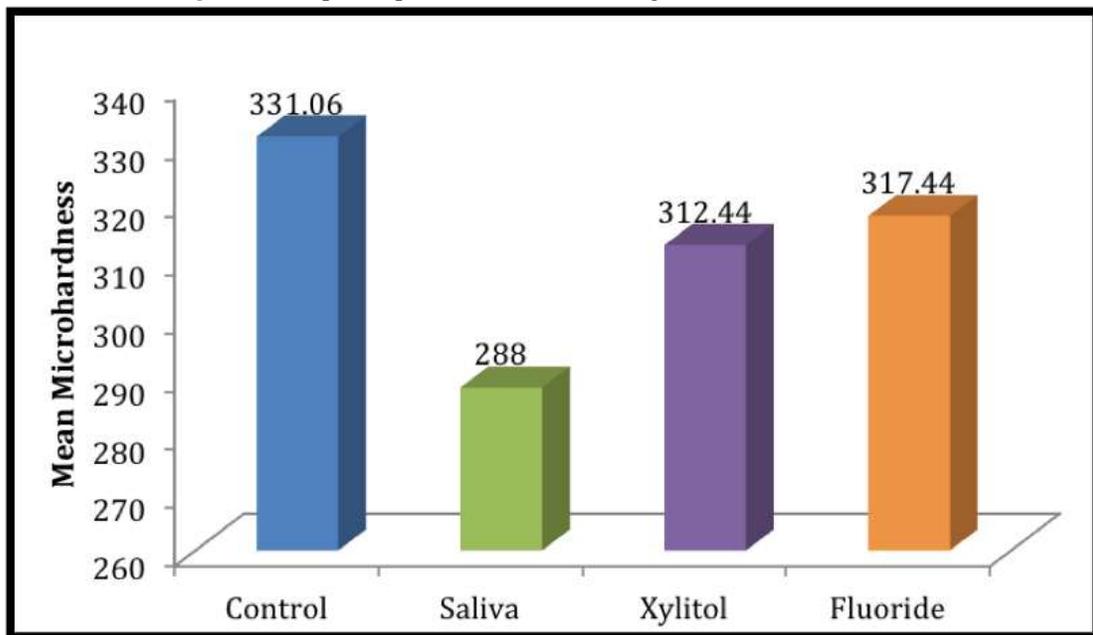
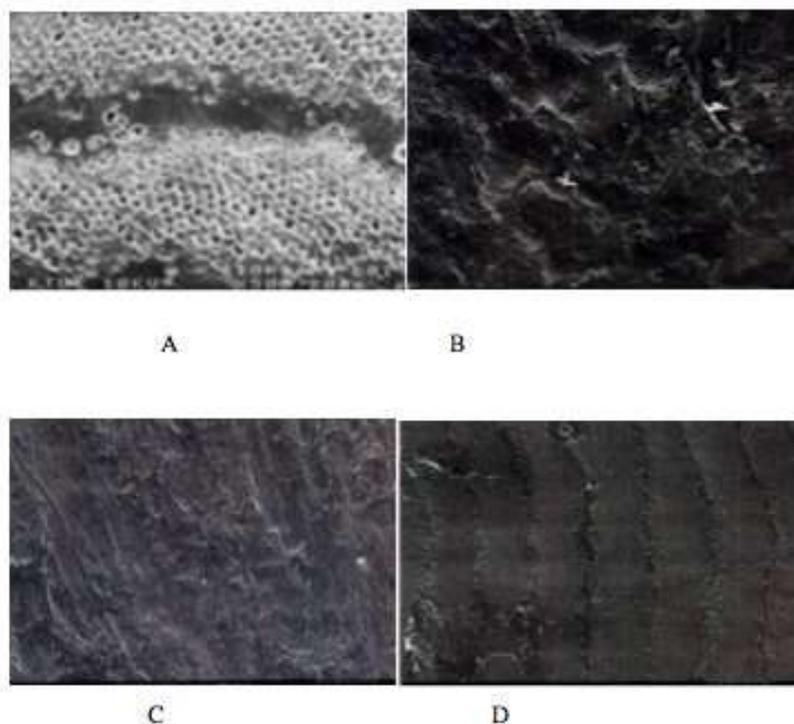


Figure 8:-SEM photomicrographs (500-times magnification)**Table 1:-ANOVA Table**

Source of Variation	MESIAL SURFACE					DISTAL SURFACE				
	Sum of Squares	Df	Mean Square	F	P-value	Sum of Squares	Df	Mean Square	F	P-value
Between Groups	15502.672	3	5167.557	200.447	<0.001	10797.125	3	3599.042	142.079	<0.001
Within Groups	1546.813	60	25.780			1519.875	60	25.331		
Total	17049.484	63				12317.000	63			

Table 2:-Tukey's Post Hoc Tests for Multiple comparisons

Group		Mean Difference	Std. Error	P-value	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Saliva	43.062	1.795	<0.001	38.32	47.81
	Xylitol	18.625	1.795	<0.001	13.88	23.37
	Fluoride	13.625	1.795	<0.001	8.88	18.37
Saliva	Xylitol	-24.438	1.795	<0.001	-29.18	-19.69
	Fluoride	-29.438	1.795	<0.001	-34.18	-24.69
Xylitol	Fluoride	-5.000	1.795	0.035	-9.74	-0.26

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