

Effect of different chocolate and candy in enamel surface loss of human permanent and primary teeth, an in vitro study

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Effect of different chocolate and candy in enamel surface loss of human permanent and primary teeth, an in vitro study

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ABSTRACT

Background. Excessive intake of candy and chocolate increases enamel surface loss among children and adults.

Purpose. To analyze the effect of different chocolate and candy in enamel surface loss of human permanent and primary teeth.

Methods. The commercial samples were exposed to a solution consisting of 5 grams of chocolate or popping candy dissolved in 2 mL of saliva twice daily for five minutes at a time for five days. After each exposure, samples were washed in distilled water for 20 seconds before the next step of the test, which involve dipping them in the artificial saliva. Artificial saliva was changed daily. As a result, the Vickers hardness test was used to retest the microhardness of each sample.

Results. There was a significant difference between the surface microhardness of primary and permanent dental enamel before and after exposure to popping chocolates and sweets. On the other hand, there was a general reduction in the surface microhardness values of the treated groups for each tested material used compared with the control group. However, when compared to the control and non-treated groups using fluoridated toothpaste, surface microhardness was considerably higher in all treated groups using fluoridated toothpaste.

Conclusion. Chocolate showed the highest enamel surface loss compared to others. Families are encouraged to exercise more control over what children and teenagers eat. It is recommended that they clean their teeth after ingesting the candy and chocolate by brushing the tooth with fluoridated toothpaste, but not directly because the enamel becomes softer when acid is removed without difficulty.

Keywords: Chocolate; Candy; Enamel surface; Permanent teeth; Primary teeth

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INTRODUCTION

In dentistry, dental erosion is a prevalent issue. Enamel loss is a biochemical process that takes place without the involvement of bacteria in which a tooth's tough surface is eroded by acidic processes [1]. Internal and environmental causes, or a mix of them, are likely to be the cause of this disease in children and young people (such as adults) [2]. The recent diet, which includes an excessive amount of juice, acidic beverages, acidic sweets, and some chewing gum with acidic centers, is the biggest risk factor for erosional processes in youngsters [3].

In the beginning, just the enamel is impacted by erosion. When dental hard tissue is subjected to an unsaturated solution, both fluorapatite and hydroxyapatite dissolve, resulting in erosion and injury to the teeth [4]. The mineral in teeth is prone to demineralization when the pH falls below the critical pH of an enamel (pH = 5.5), but they are re-mineralized when the pH rises just above the critical pH level [5,6].

However, when it comes to prevention, there are natural ways to help stop tooth loss. These include avoiding sticky and sweet foods and drinking lots of water; brushing your teeth correctly and using fluoride toothpaste; visiting the dentist and getting sealants and fluorides applied topically (if there are deep pits and fissures); and maintaining a diet rich in foods that are good for your teeth, such as low-fat milk, cheese, curd, soy milk, tofu, nuts, eggs, dark green leafy vegetables, lean meat, fish, beans, and chocolates [7]. Chocolates are of three kinds: milk chocolates, dark chocolates, and white chocolates. Dark chocolate is one of many varieties of chocolate. The cocoa bean is the main healthful ingredient in chocolate; nevertheless, not all chocolate varieties are tooth-friendly. Consequently, chocolate is healthier as a result. Numerous nutrients and antioxidants included in cocoa beans are beneficial for the entire body, not just the mouth and teeth. Theobromine is another phenolic substance present in cocoa husk beans. Theobromine is one such main constituent in cocoa beans and is found in higher concentrations in the dark than in milk chocolate [8].

Numerous studies have revealed that these ingredients have anti-cariogenic effects. These substances have been shown to inhibit bacterial adhesion to tooth surfaces, which lowers the number of germs [8,9]. When exposed to liquids containing chocolate or sweets, the tooth enamel's hardness decreased and the surface roughness increased, which led to tooth erosion.

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Only the candy's pH fell below the critical enamel pH. Families are encouraged to exert more control over what kinds of candies and chocolates their children and teenagers have access to [3].

A further worrisome dimension to the role that sweets play in tooth decay and destruction is the rapid rise in the use of various food acids as flavoring agents, which can be attributed to the introduction and widespread consumption of acidic candies, which are primarily consumed by young adults, teens, and small children [10]. It's intriguing because sour candy contains sizable amounts and varieties of acids, necessitating investigation into how they impact tooth enamel [11]. A noteworthy public health concern is the general increase in acidic (sour) candy consumption among children and teenagers [12].

It's also been noted that some candies degrade tooth enamel more than acidic beverages, but the data is still insufficient to determine how much of an impact candies have [13]. Various food intakes on a daily basis could potentially impact tooth mechanics, shape, and surfaces. Accordingly, we sought to evaluate the effect of different chocolates and candy on the enamel surface loss of human permanent and primary teeth; fluoride is used in the current study as a preventive method.

5 Materials and Methods

In this in vitro study, two distinct sweet types and four distinct chocolate varieties that are often seen in the Mosul market were used. Table 1 in the context of the factory report lists the different types of candies and chocolate, composites, and the country of origin of the components. After the release of ethical agreement from the scientific committee in the College of Dentistry (University of Mosul) was attained to achieve the study, fifty-six healthy primary incisor teeth and permanent incisor teeth were carefully chosen based on the inclusion criteria involving extracted sound permanent premolar and deciduous incisors of less than 3 months post-extraction with intact buccal and labial enamel surfaces. To get rid of any saliva, blood, or even other debris, the teeth were cleaned. After being scrubbed with pumice, they were immersed in the serum as the experiment began. The samples were glued to the resin composites so that enamel would appear. After that, sandpaper was used to smooth off the outside of the samples' enamel (600 grit and then 1200 grit). Then, using a Vickers hardness tester, the enamel microhardness of each sample was determined [3,14].

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This sample was subdivided into subgroups, which contain: the control (artificial saliva) group and 12 experimental groups (six before and six after exposure to toothpaste for 5 minutes by fine brush once a day for 5 days): A (jelly beans), B (white chocolate), C (gumballs), D (milk chocolate), E (dark chocolate), and F (family milk chocolate with chocolate filling) (chocolate beans). Each candy and bar of chocolate was crumpled with a pestle before the test, and 5 grams of each were dissolved in 2 ccs of synthetic saliva at room temperature [3]. At that point, their pH was measured with a pH meter, and sugar per 100 grams for each kind was recorded in the factory report in Table 1. The samples were subjected to a solution consisting of 5 grams of chocolate or popping candy diluted in 2 mL of saliva twice, for five minutes each time, for five days. Following every exposure, samples were washed in distilled water for 20 seconds before the subsequent step of the test, which involved dipping them in the artificial saliva. Artificial saliva was replaced every day. Therefore, each sample's microhardness was once again measured using the Vickers hardness test. To analyze the data, Armonk, New York independent and paired t-tests, the Tukey test, and an ANOVA were utilized [3,14].

RESULTS

All of the materials evaluated in the current study had PH values higher than 7, except jelly beans and chocolate beans, which had PH values of 6.8 and 4.5, respectively, underneath the critical pH of enamel (5.5). primary and permanent, as illustrated in Table 2. The results of this investigation showed that the surface hardness value of the enamel of the permanent and primary teeth differed significantly before and after exposure to popping chocolates and candy, respectively (paired t-test, $P < 0.001$). Jelly beans (A), chocolate beans (F), milk chocolate (D), white chocolate (B), gumballs (C), and dark chocolate (E) were the substances that caused the greatest alteration in the surface microhardness of the enamel of primary teeth. On the other hand, there was a general reduction in the surface microhardness values of the treated groups for each tested material used compared with the control group, while surface microhardness was significantly increased in all treated groups with fluoridated toothpaste compared with the untreated groups with fluoridated toothpaste and the control group, as shown in Table 3 for primary and permanent teeth.

DISCUSSION

Due to the excessive intake of candy and chocolate in recent years, enamel surface loss has increased among children, teenagers, and adults [11-15]. In the current study, all groups saw a reduction in the enamel's surface microhardness after being subjected to sweet or chocolate-flavored beverages. Hardness, a crucial mechanical property of matter, is the ability of a substance or surface to resist piercing or indentation [16]. An increase in hardness improves a material's general resilience to wear, contact, and erosion with other materials. This indicates that enamel with a higher mineral concentration wears less [16]. For the claim that exposure to an acidic solution causes the enamel surface to initially lose its hardness value before enamel tissues degrade and eventually disappear [17].

We assessed the primary and permanent enamel hardness using the Vickers method following the teeth's exposure to chocolate and candy [16]. Any diet with a pH lower than 5.5 may contribute to or enhance enamel surface loss because jelly beans have a pH (4.5) that is below the enamel critical pH (5.5), which is thought to be the cause of the high drop in microhardness of the enamel surface. Clinical research has shown, and this is particularly significant, that salivary pH can drop as low as 2.96 minutes after eating candy [11,15]. Dental enamel is affected by variations in the oral environment and decreased pH near the surface of enamel produced by bacterial metabolism. In this case, demineralization is due to the change of the inorganic constituent $[Ca_5(PO_4)_3(OH)]$, thus changing physical properties, for example, hardness. When available Ca^{2+} and PO_4 -ions interrelate with the dental structure, a homeostatic mechanism takes place, improving the physical properties of demineralized tissues [18].

Acidic ingredients found in sweets include tartaric, malic, phosphoric, citric, oxalic, fumaric, and carbonic acids. This indicates that jelly beans may be more erosive than chocolate, which means they could harm a tooth's enamel more severely. Current study by Sultan et al. (2022) [19] and El-Marakby from 2018 [20]. Additionally, prior research has demonstrated that, similar to gumballs, which have a pH of 6.8, enamel may dissolve at pH ranges of 5.2-2.9% and 6.0-6.7, respectively. 21,22 Even though the pH of the other materials under examination fell short of the enamel critical pH, enamel surface loss is still influenced by pH, exposure time, frequency, acid type, acid concentration, and sugars contained, which enhance bacteria to yield acid and lower the pH.23 The results are consistent with other studies [24,26].

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Conversely, the least damaging foods for teeth include white chocolate, dark chocolate, milk chocolate, and chocolate beans because they contain another chief good constituent, such as cocoa (*Theobroma cocoa*), which contains theobromine, cocoa butter, and numerous minerals that harden the tooth enamel and decrease the risk of demineralization and dental erosion [9]. Moreover, those kinds of chocolate have calcium, which is considered an important mineral for tooth remineralization [27]. To this point, samples that were also exposed to water showed signs of demineralization. Deionized water is reportedly very aggressive. This could be explained by the absence of calcium, which serves as a protective agent against erosional attacks [28].

They found in multiple investigations that the demineralization and enamel surface loss of primary teeth are different from those of permanent teeth. The most noticeable differences between primary and permanent teeth are found in their anatomical makeup, with primary teeth having a thinner and more porous enamel layer and being smaller overall. While the enamel prisms of both types of teeth are similar, primary teeth contain prisms that are smaller, more curved, and more widely distributed. Their hydroxyapatite crystals also differ greatly from one another [29]. Crystals of hydroxyapatite, which are imperfect forms found in both permanent and primary enamel, are essentially composed of calcium (Ca^{2+}), phosphate (PO_4^{3-}), and hydroxyl (OH^-) ions, along with some "impurity" ions, such as sodium (Na^+), fluoride (F^-), and carbonate (CO_3^{2-}). These ions are arranged in a crystalline structure with the simplified chemical formula: $\text{Ca}_{10-x}\text{Na}_x(\text{PO}_4)_6-y(\text{CO}_3)_z(\text{OH})_2-u\text{F}_u$. Carbonate (CO_3) is a significant impurity ion that distinguishes permanent enamel from primary enamel [30].

The results of this study showed the greatest alteration in the surface microhardness of the enamel of primary teeth. Numerous studies have shown that permanent enamel is much more erosion-resistant than primary enamel. 31 Conversely, Carvalho et al. (2017) showed that the quantity of surface hardness reduction in permanent teeth and primary teeth was not significantly different [25].

Topical fluoride has had an important effect on decreasing the risk of enamel surface loss and dental caries. The major method recommended for preventing or treating early lesions is the use of various toothpaste types, which are accompanied by mouth rinses and gels with strong active biological components. In addition to other essential elements, several types of toothpaste also contain active compounds. Fluoride is the most prevalent of the altered active

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composites that support the improvement of enamel remineralization in toothpaste [32]. In 2020, Zanatta et al. released a meta-review just on fluoride composites' capacity to reduce erosive tooth wear [33]. On the other hand, another study assessed the in vivo resistance of dental enamel exposed to acid attack both before and after the use of various fluoride agents, concluding that the treatments created an increased resistance to acid attack. By making enamel harder and more stable and preserving the apatite structure, fluor has an impact on the chemical and physical characteristics of apatite minerals [34]. Evidence suggests that an absence of fluoride can cause tooth demineralization, even if a person takes care of their teeth [34]. Studies showed that, in comparison to untreated teeth, fluoride-treated teeth were much more acid-resistant [17,36].

Comparing human primary and permanent teeth, we discovered that primary enamel released more calcium and phosphate than permanent enamel, both in native samples and during pellicle formation. These observations, while not usually significant, indicate that primary enamel is more easily eroded than enamel from permanent human teeth [37]. In comparison to human permanent teeth, this also shows the lower degree of mineralization of primary enamel, together with higher porosity and susceptibility to erosive enamel flaws. Human primary enamel tended to erode more quickly than permanent teeth, but dentin exhibited the opposite pattern [37].

A long-term in vitro erosion study (5–15 days) that exposed primary and permanent enamel to low-pH fruit drinks had similar findings; however, the findings explained by the structural variations in enamel between primary and permanent teeth also validate the greater erosion vulnerability of primary teeth [37]. The findings demonstrated that the microhardness of tooth enamel was significantly reduced by these sweets and chocolates, with the primary teeth seeing a larger drop in microhardness than the permanent teeth. Some distinctions exist between primary and permanent enamel teeth, including variations in mineralization and thickness, which may have an impact on the outcome [38]. Other methods for the detection of enamel surface loss structure defects, such as microhardness, can only examine flat-parallel ground specimens, which precludes the examination of native hard substance specimens. Because of their curved shape and reduced stress, incisors wear down less than molars [37]. Moreover, those kinds of chocolate have calcium, which is considered an important mineral for tooth remineralization [27]. To this point, samples that were also exposed to water showed signs of demineralization. Deionized water is reportedly very aggressive. This could be explained by the absence of calcium, which serves as a protective agent against erosional attacks [28].

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When Lazzaris et al. (2015) investigated the acidity and erosive potential of commercially available candies, they also found that 3 jelly beans had a pH 4 lower than the enamel's critical pH and that the pH of the candies varied in flavor [39]. Additionally, this study is compatible with Leelavathi and Chaly's work, which evaluated the pH of lollipops with various tastes. In this study, the effects of candy and chocolate on teeth were examined, and it was shown that jelly beans had a greater drop in microhardness since erosion is also influenced by pH, frequency, exposure duration, and acid type and concentration [40]. Although there hasn't been any particular research on how tooth hardness is affected by dark chocolate, the analysis of theobromine—the main ingredient in cocoa—for enamel and dentin matches those of previous studies that looked into the same topic. Enamel hardness was elevated by theobromine, and the inorganic minerals had a direct impact on this process. When it comes to cocoa powder, milk chocolate has a range of 1.2% to 2.4%, but dark chocolate has a higher theobromine level. 5 Furthermore, unsaturated free fatty acids, such as oleic and linoleic acids, have antibacterial properties against Streptococcus mutans. A medium with anti-carcinogenic capabilities has been developed. Prior research into the mechanism underlying theobromine's anti-cariogenic characteristics has demonstrated the creation of an appetite-structured medium that facilitates the remineralization process, which rehardens the tooth surface [23,42].

Because milk chocolate has a lower melting point at body temperature than dark chocolate, it melts more easily on the tongue and has less hardness than dark chocolate due to its combination of cocoa butter and milk fat (milk butter and milk cream) [43]. Theobromine, a substance found in dark chocolate made from Theobroma cacao, is known to increase tooth [Type text]

enamel hardness and reduce the risk of dental cavities [23]. Chemically speaking, theobromine has the formula $C_7H_8N_4O$. It has been shown that theobromine compounds can raise the tooth enamel surface's hardness by encouraging interstitial contacts between hydroxyapatite crystals (HA) and theobromine on the enamel surface [23,42]. Typically, between 50 and 90 percent of the ingredients in dark chocolate are cocoa beans. They are also a rich source of minerals like iron, magnesium, zinc, copper, potassium, selenium, phosphorus, and manganese [23,44], as well as antioxidants like tannins, polyphenols, and flavanols (which include epicatechins, monomers, and catechins) and valuable components like theobromine (1.2–2.4%) [23]. Some earlier research has found that the minerals included in dark chocolate include iron, magnesium, zinc, copper, potassium, selenium, phosphorus, and manganese. These minerals are crucial for creating a stronger tooth structure. Inorganic minerals were directly linked to improving the hardness of the enamel, and theobromine further enhanced its hardness [9,23,42]. Candy is a common product that is enjoyed by both young and old. Its main component, sugar, provides an immediate energy boost. It is typically combined with different colors and flavors to provide a visually appealing and sensual experience. Among the more than 2000 varieties, flavors like caramel, chocolate, peppermint, butterscotch, and vanilla are the most widely consumed [45].

Brand name specifications may not be employed unless the specific brand name, product, or feature is required by the government, and market research shows that similar products from other companies or products without the specific feature, do not meet, or cannot be modified to meet, the needs of the agency. Unfortunately, direct comparisons that evaluate enamel surface loss models by contrasting not just species but also permanent and primary dentition are uncommon, and the experimental criteria used in these comparisons are frequently extremely dissimilar.

When fluoride is applied under acidic conditions, the enamel layer (hydroxyapatite) partially demineralizes and dissolves fluoride's biological effects, including its antibacterial action and a decrease in the attachment of bacteria to fluoridated tooth surfaces [46]. Fluoride may also have chemical actions that contribute to its protective effects. As a result, partial demineralization (partially dissolves) of the tooth enamel (hydroxyapatite) and reprecipitation of fluoride-containing minerals are the chemical effects of fluoride treatment. However, in most situations, there is restricted incorporation of fluoride into the tooth hydroxyapatite to presumably generate fluor-hydroxyapatite, even at very high concentration levels (e.g., fluoride

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gel; not toothbrushes or mouthwashes). The fluoride treatment reduced the demineralization of the teeth. It has been extensively proven that fluoride has a remineralizing and protecting effect. It is critical to keep in mind that the action of fluoride, which is frequently produced from saliva, depends on calcium and phosphate ions [2]. which is followed by the use of fluoride-containing minerals. This fluoride-containing layer thickens with fluoride concentration and is also helped by a low pH level. There is a chance for many hundred nanometers [2]. There have been theories regarding

This study's weakness was that it only looked at the primary and permanent incisors. It is advised that future research examine the impact of the chocolate and candy on the extracted hidden posterior teeth. It is critical to keep in mind that the action of fluoride, which is frequently produced from saliva, depends on calcium and phosphate ions.

Chocolate showed the highest enamel surface loss compared to others. Families are encouraged to exercise more control over what children and teenagers eat. It is recommended that they clean their teeth after ingesting the candy and chocolate by brushing the tooth with fluoridated toothpaste, but not directly because the enamel becomes softer when acid is removed without difficulty.

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Table 1. Types, Compounds, PH, Sugar content, and the manufacturing country of the tested materials.

| Materials | Composition | pH | Sugar | Country |
|-----------|-------------|----|-------|---------|
|-----------|-------------|----|-------|---------|

[Type text]

| | | | | |
|--|---|-----|------|---------|
| A (Jelly beans) | Glucose syrup, sugar, Cider Center 7.5 %, Halal bovine gelatin sourced from Turkey, Citric acid antacid, Natural cola colors, and Polishing agent (Carnauba wax, Beeswax). | 4.5 | 47.3 | Turkey |
| B (White chocolate) | Sugar, cocoa butter, whole milk powder, skimmed milk powder, stabilizer (calcium carbonate), emulsifiers (sunflower lecithin, polyglycerol polyricinoleate), flavoring (vanillin). It May contain hazelnut, pistachio, almond, and wheat). | 7.4 | 50.8 | Turkey |
| C (Gum balls) | Sugar, gum base, glucose syrup, flavors, glycerin, lecithin, citric acid, food color, pectin, and water. | 7.0 | 50.0 | Iran |
| D (Milk chocolate) Family milk chocolate with chocolate filling | Family milk chocolate (70 %), sugar, cocoa butter, cocoa mass, skimmed milk powder, whey powder (milk)fat, mineral (calcium carbonate) emulsifier (sunflower lecithin, polyglycerol polyricinolate), flavoring (vanillin), white chocolate (30 %) (sugar, cocoa butter, whole milk powder, skimmed milk powder, mineral (calcium carbonate), emulsifier (sunflower, lecithin, polyglycerol polyricinolate), flavoring (vanillin). traces: wheat (gluten), almond, hazelnut, pistachio). Cocoa solids in milk chocolate minimum:29%. | 7.6 | 55.3 | Turkey |
| E (Dark chocolate) | Sugar, cocoa paste, cocoa butter, butterfat emulsifier, lecithin's (soya), vanilla extract, and cocoa solids: 50% minimum may contain traces of peanuts, nuts, gluten, and egg | 7.2 | 47.0 | Germany |
| F(Chocolate beans) | Sugar, Cocoa Powder, skimmed milk powder, Hydrogenated Palm kernel oil, listen (E-322), Vanilla, carnauba (E- 903), White Dextrin Food colors (E-110, E-129, E-133, E-155, E-171). | 6.8 | 70.1 | Lebanon |

Table 2. Comparison of the microhardness before and after effect of chocolate and candies in each group (n=8) (paired t-test) (primary and permanent teeth).

| Groups | Microhardness | Mean+ SD (Primary Teeth) | Mean+ SD (Permanent Teeth) | paired t-test |
|-----------------------------|---------------|--------------------------|----------------------------|-----------------|
| Control (Artificial Saliva) | before | 337.16±11.83 | 338.0+ 1.58 | <i>P</i> <0.001 |
| | after | 335.83±20.10 | 337.20+ 1.30 | |
| (A) With toothpaste | before | 330.0±12.64 | 335.20+1.92 | <i>P</i> <0.001 |
| | after | 256.66±15.05 | 264.40+0 .89 | |
| (A) Without toothpaste | before | 330.0±12.64 | 335.20+1.92 | <i>P</i> <0.001 |
| | after | 224.16±9.17 | 234.0+0 .70 | |
| (B) With toothpaste | before | 333.33±10.32 | 335.20+0.83 | <i>P</i> <0.001 |
| | after | 262.50±11.72 | 266.80+1.30 | |
| (B)Without toothpaste | before | 333.33±10.32 | 335.20+0.83 | <i>P</i> <0.001 |
| | after | 235.83±13.57 | 239.60+1.14 | |
| (C) With toothpaste | before | 336.66±5.16 | 337.60+1.14 | <i>P</i> <0.001 |
| | after | 269.16±22.89 | 272.0+ 1.58 | |
| (C) Without toothpaste | before | 336.66±5.16 | 337.60+1.14 | <i>P</i> <0.001 |
| | after | 240.83±11.14 | 246.0+ 2.54 | |
| (D) With toothpaste | before | 333.33±8.16 | 336.60+ 2.07 | <i>P</i> <0.001 |
| | after | 265.83±16.25 | 270.0+ 1.58 | |
| (D) Without toothpaste | before | 333.33±8.16 | 336.60+ 2.07 | <i>P</i> <0.001 |
| | after | 234.16±4.91 | 241.60+ 2.07 | |
| (E) With toothpaste | before | 335.0±10.48 | 337.0+1.58 | <i>P</i> <0.001 |
| | after | 267.50±18.37 | 272.40+ 1.51 | |
| (E) Without toothpaste | before | 335.0±10.48 | 337.0+1.58 | <i>P</i> <0.001 |
| | after | 239.16±24.16 | 245.60+ 1.14 | |
| (F) With toothpaste | before | 338.42±10.67 | 339.60+ 1.14 | <i>P</i> <0.001 |
| | after | 270.0±11.54 | 274.80+1.48 | |
| (F) Without toothpaste | before | 338.42± 10.67 | 339.60+ 1.14 | <i>P</i> <0.001 |

[Type text]

| | | | | |
|--|-------|-------------|-------------|--|
| | after | 238.57±8.99 | 244.40±1.14 | |
|--|-------|-------------|-------------|--|

Table 3. Comparison of the effect of candy and chocolate on the microhardness of the primary and permanent teeth enamel groups.

| Groups | Microhardness (Mean±SD) (Primary Teeth) | Microhardness (Mean±SD) (Permanent Teeth) |
|-----------------------------|--|--|
| Control (Artificial Saliva) | 1.33±18.34 | 0.80±0.44 |
| (A)With toothpaste | 73.33±20.65 | 70.80±1.30 |
| (A)Without toothpaste | 105.83±14.97 | 101.20±2.16 |
| (B)With toothpaste | 70.83± 14.28 | 68.40±0 .89 |
| (B)Without toothpaste | 97.50±16.65 | 95.60±0.54 |
| (C) With toothpaste | 67.50±22.74 | 65.60±1.51 |
| (C) Without toothpaste | 95.83±13.57 | 91.60±2.60 |
| (D)With toothpaste | 67.50±16.65 | 66.60±1.51 |
| (D) Without toothpaste | 99.16±8.01 | 95.0±4.12 |
| (E)With toothpaste | 67.50±16.65 | 64.60±2.30 |
| (E) Without toothpaste | 95.83±22.45 | 91.40±1.51 |
| (F)With toothpaste | 68.42±20.34 | 64.80±1.30 |
| (F) Without toothpaste | 99.85±15.17 | 95.20±1.30 |

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