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Assessing the Impact of Varied Dark Chocolate Concentrations on Enamel and Dentine Microhardness

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Abstract: (1) Background: The objective of the current investigation was to determine how different dark chocolate concentrations impacted the enamel and dentine microhardness. (2) Methods: Twenty noncarious premolar teeth extracted for orthodontic reasons were used in this study. Each tooth was divided mesiodistally to obtain 40 specimens of enamel and dentine 4 × 4 mm. Initial and final assessments of the surface microhardness of the enamel and dentine were created using a Digital Micro Vickers Hardness Tester (Laryee, Beijing, China) under (0.24 N load for 15 s). Four random groups (n = 10) were created: G1 (control, immersed in artificial saliva), G2 (70%), G3 (85%), and G4 (100% dark chocolate). Each sample underwent four cycles of 60-s immersion in suggested concentrations, followed by 30 min in artificial saliva. The attained values underwent statistical analysis employing SPSS version 26, with the utilization of T-tests and ANOVA. (3) Results: The results revealed a significant, incremental increase in the average microhardness values for enamel, specifically 61.72 ± 22.729, 64.17 ± 23.397, and 109.15 ± 34.625, and for dentin from 57.91 ± 8.332 to 72.29 ± 2.752 and ultimately reaching 88.67 ± 8.602, corresponding to the escalating concentrations of dark chocolate (70%, 85%, and 100%) ($p < 0.001$). (4) Conclusions: Immersing the specimens in different concentrations of dark chocolate had a significant positive impact on the microhardness of both enamel and dentine.

Keywords: enamel; dentin; chocolate; artificial saliva; hardness; dentistry

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1. Introduction

Chocolate is one of those junk food products, and a frequent misconception holds that it is the primary cause of tooth cavities, which is misleading [1]. As a natural food, dark chocolate is considered healthy and beneficial [1]. It comes from cacao, which is another name for cocoa. Dark chocolates (approximately 50–60%) have a semisweet flavor, and extra dark chocolates (75–90%) have a bittersweet flavor. Dark chocolate has a variety of names, including plain chocolate, black chocolate, and sour chocolate [2].

It is used as a replacement, along with cocoa or chocolate. Chocolate (Theobroma cacao) is a culinary product manufactured from cacao seeds [3]. Due to its sweet flavor, individuals think that chocolate has a significant impact on the growth of dental caries [3]. In reality, the chocolate product's high sugar level may have contributed to the consequences of tooth decay [3].

Dark chocolate, derived from Theobroma cocoa, includes theobromine, a component known to enhance tooth enamel hardness and lower the likelihood of dental caries [4]. Theobromine (3, 7-dimethylxanthine) is the main alkaloid generated from the "theobroma cacao plant." [5]. It is also found in cocoa leaves, in lower concentrations [6]. Chocolates, tea, and other foods all contain theobromine, a bitter, crystalline, water-soluble substance [5].

It simply differs from caffeine by one methyl group [7]. Theobromine is represented chemically by the formula $C_7H_8N_4O$ [8]. Through promoting interstitial interactions between the hydroxyapatite crystals (HA) and theobromine on the enamel surface, it has been demonstrated that theobromine compounds can increase the hardness of the tooth enamel surface [6].

Dark chocolate typically comprises 50–90% cocoa beans, a rich source of essential components including theobromine (1.2–2.4%), various minerals like iron (Fe), magnesium (Mg), zinc (Zn), copper (Cu), potassium (K), selenium (Se), phosphorus (P), and manganese (Mn), as well as valuable antioxidants such as tannins, polyphenols, and flavanols (comprising monomers, epicatechins, and catechins) [9].

Advantages of consuming dark chocolate for health: it may lower the risk of atherosclerosis by restoring the flexibility of arteries and preventing the adhesion of white blood cells [10]. Cocoa beans, rich in flavanols, hold the potential to mitigate cardiometabolic disorders [11]. Dark chocolate, abundant in polyphenols, can enhance endothelial function and reduce blood pressure in individuals in the initial stage of hypertension [12]. Dark chocolate enhances circulation, offering protection against type II diabetes mellitus while promoting the health of blood vessels. The flavonoids in dark chocolate contribute to reducing insulin resistance, allowing cells to function normally and regain the ability to efficiently utilize insulin in the body [13]. Dark chocolate enhances cognitive activity by boosting blood flow to both the brain and the heart. Its essential compound content stimulates brain function, positively affecting cognitive health. Phenylethylamines in dark chocolate trigger the release of endorphins from the brain, leading to increased alertness [14]. With a caffeine content lower than that of coffee, dark chocolate serves as a mild stimulant, promoting alertness, aiding in weight loss, enhancing sports performance, boosting brain functions, and assisting in memory retention [15]. Dark chocolate is rich in antioxidants, which play a crucial role in protecting the body against free radicals—uncharged molecules that can cause oxidative stress and damage to cells. This protective effect extends to guarding against various types of tumors and cancers, as well as reducing the visible signs of aging [16].

Thus, the objective of this in vitro investigation was to assess the microhardness of dental enamel and dentin following immersion of the specimen in various dark chocolate concentrations. The study's null hypothesis was that there was no impact of the dart chocolate on enamel or dentin hardness.

2. Materials and Methods

2.1. Materials

Dark chocolate with varying cocoa concentrations of 70%, 85%, and 100% (Excellence Lintd, Oloron, France) commercially available, was chosen for examination. These chocolates were acquired from supermarkets in Erbil, Iraq. The firm, dark chocolate samples were stored in a refrigerator at 4 °C. Prior to analysis, all samples were chopped into small pieces and were melted using a double boiler. Utilizing the stovetop for melting ensured that the chocolate did not burn, leading to uniform melt with a silky-smooth texture, ready for the immersion of the specimens.

2.2. Artificial Saliva Preparation

An artificial saliva solution was formulated by combining the following components: 500 mL of distilled water, 20 g of potassium chloride, 0.843 g of sodium chloride, 0.051 g of magnesium chloride, carboxymethyl cellulose, 20 mL of tricalcium phosphate, and 0.05 M sodium hydroxide. The inclusion of sodium hydroxide was intended to regulate and maintain a pH level of 6.8 in the solution [17].

2.3. Specimen Preparation

In this study, a total of 20 premolar teeth were extracted for orthodontic reasons and were free from cavities. The premolars' intactness was the inclusion criterion, as well as

teeth with cracks, caries, or other defects not included in the study. A periodontal scalar was used for eliminating organic debris, and prophylaxis was carried out using a rubber cup and pumice slurry (a mixture of pumice pastes and water). A stereomicroscope (SMZ 25 Nikon, Tokyo, Japan) was used for evaluating the teeth at ×4 magnifications in order to exclude teeth with hypoplastic defects or a crack.

Prior to the start of specimen processing, the chosen teeth were kept in a 0.1% thymol solution as a disinfectant. Following their preparation, the specimens were placed in deionized water for storage. These specimens were prepared by sectioning the teeth horizontally with the aid of a device cutter (PRIME 407 Ltd., Hebei, China) to separate the crown from the root. Two specimens of enamel and dentine from each tooth were obtained by sectioning the crowns vertically in the mesiodistal direction. The roots were disposed of. The prepared specimens were cut with a dental lab diamond disc double-sided grit cutting disc under cooling to achieve blocks measuring 4 × 4 mm. Acrylic resin (Shanghai New Century Dental Materials Co., Ltd., Shanghai, China) was used to embed these blocks with the aid of a device (SJK Dental Corporation Limited, Shanghai, China); subsequently, they underwent a polishing process through a polishing machine (PRIME 407 Ltd., Hebei, China) using a cyber denture adjustment kit from coarse, medium, and fine at 5000–7000 rpm, providing a surface that is flat, regular, and smooth [18].

2.4. Microhardness Assessment Measurement

For each specimen, an initial microhardness assessment was conducted, both on the enamel surface and within the dentin, with a digital micro-Vickers Hardness Tester (Laryee, Beijing, China) using a 0.24 N load for 15 s. For each specimen, three dents were created using a diamond indenter to impress a small area on the tooth surface, and the initial microhardness mean was recorded. After finishing the specimens, they were placed in deionized water for storage. The 40 specimens were divided at random into four groups (n = 10). Figure 1: G1-10 specimens immersed in artificial saliva (control group), G2-10 specimens immersed in 70% dark chocolate, G3-10 specimens immersed in 85% dark chocolate, and G4-10 specimens immersed in 100% dark chocolate. After 60 s of immersion in each concentration proposed, each group was immersed in artificial saliva for 30 min. This process was repeated a total of four times [18]. Then, the final average microhardness was calculated for each group in a similar way used to record the initial microhardness.

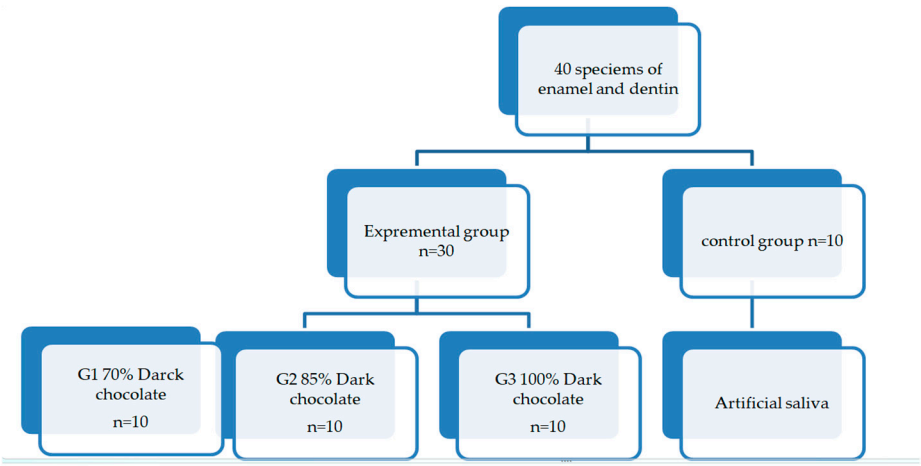

Figure 1. Schematic diagram for the studied group.

2.5. Statistical Analysis

As part of the descriptive statistics, a parametric test was employed to analyze the collected sample study and to assess differences between and within groups. The paired sample t-test was carried out for within groups, and One-Way ANOVA was utilized to compare the different types of the dark chocolate. Additionally, normality assumption was

assessed with Shapiro-Wilk technique and the chosen threshold for statistical significance was set at a p-value < 0.05. All statistical reports in this study were generated using R version 4.3.2 and SPSS (version 26 for Windows), a software program from IBM in New York, NY, USA.

3. Results

3.1. Enamel Microhardness

Figure 2 depicts a bar chart illustrating microhardness average mean values for enamel both initially and after immersing specimens in various concentrations of dark chocolate. The study groups include G1 (control group), exposed to artificial saliva, which exhibited a mean difference ranging from 236.09 to 243.34. In comparison, G2 (70% dark chocolate), G3 (85% dark chocolate), and G4 (100% dark chocolate) displayed average mean differences spanning from 235.09 to 296.81, 235.53 to 299.70, and 235.95 to 345.10, respectively.

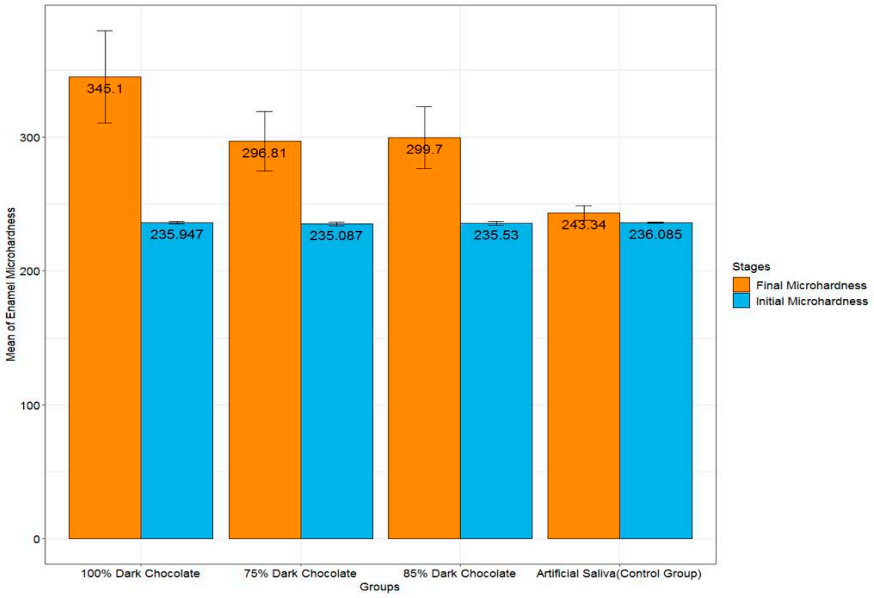

Figure 2. Represents an error bar chart, showcasing the standard deviation and mean values of enamel microhardness at both the initial and final stages.

Table 1 displays the mean and standard deviation values, along with the standard deviation difference and the difference of mean denoted as DF. The findings indicate a noteworthy, gradual increase in the average microhardness values for enamel, specifically 61.72 ± 22.729, 64.17 ± 23.397, and 109.15 ± 34.625, corresponding to the escalating concentrations of dark chocolate (70%, 85%, and 100%). Significantly discernible differences were observed between the initial and final phases within each group, with a p-value < 0.05 indicating statistical significance.

Table 1. Enamel microhardness comparison between initial and final phases in each group.

Enamel Microhardness Mean Value and SD								
Group	**Initial Microhardness**			**Final Microhardness**				
	N	**Mean**	**SD**	**Mean**	**SD**	**DF**	**SD**	***p*-Value**
Artificial saliva (control group)	10	236.09	0.533	243.34	5.290	7.25	5.389	0.002
75% Dark chocolate	10	235.09	1.298	296.81	22.253	61.72	22.729	<0.001
85% Dark chocolate	10	235.53	1.349	299.70	23.118	64.17	23.397	<0.001
100% Dark chocolate	10	235.95	0.875	345.10	34.681	109.15	34.625	<0.001

N = number of samples in each group, SD = standard deviation, DF = difference of mean.

3.2. Dentin Microhardness

Figure 3 presents a bar chart illustrating the average mean microhardness values for dentine, both initially and after immersing specimens in various concentrations of dark chocolate. The study groups consist of G1 (the control group) exposed to artificial saliva, which demonstrated a mean difference ranging from 51.31 to 60.95. In contrast, G2 (70% dark chocolate), G3 (85% dark chocolate), and G4 (100% dark chocolate) exhibited average mean differences ranging from 51.79 to 109.70, 51.81 to 124.10, and 51.53 to 140.20, respectively.

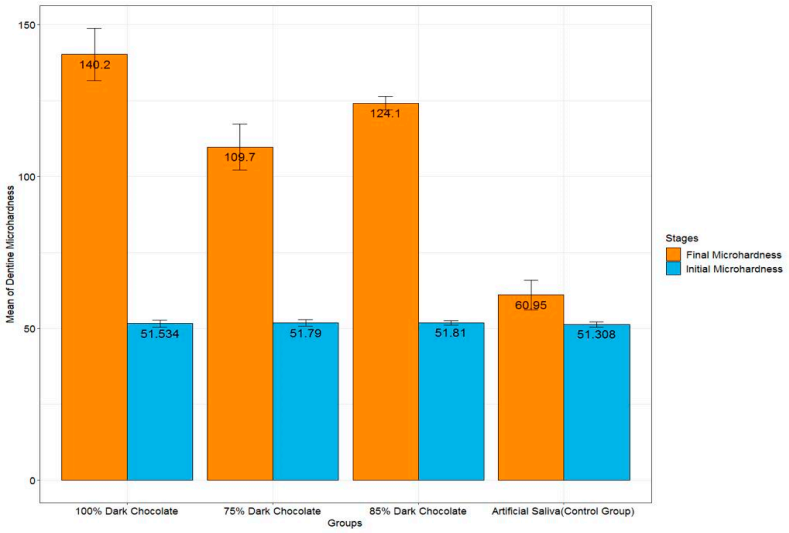

Figure 3. Represents an error bar chart, showcasing the standard deviation and mean values of dentin microhardness at both the initial and final stages.

In Table 2, the mean and standard deviation values, along with the standard deviation difference and the difference of mean denoted as DF, are presented. The results reveal a notable and gradual increase in the average microhardness values for dentine. This increase progressed from 57.91 ± 8.332 to 72.29 ± 2.752, ultimately reaching 88.67 ± 8.602, corresponding to the escalating concentrations of dark chocolate (70%, 85%, and 100%). Significant and discernible differences were observed between the initial and final phases within each group, with a p-value < 0.05 indicating statistical significance.

Table 2. Dentine microhardness comparison between initial and final phases in each group.

Group	Dentine and SD							
	Initial Microhardness			Final Microhardness				
	N	Mean	SD	Mean	SD	DF	SD	*p*-Value
Artificial saliva (control group)	10	51.31	0.904	60.95	4.910	9.64	5.083	0.0002
75% Dark chocolate	10	51.79	1.036	109.7	7.602	57.91	8.332	<0.001
85% Dark chocolate	10	51.81	0.774	124.10	2.183	72.29	2.752	<0.001
100% Dark chocolate	10	51.53	1.125	140.20	8.664	88.67	8.602	<0.001

3.3. Comparision of Enamel and Dentin Microhardness between Pairs of Groups

Tables 3 and 4 show the comparison between different concentrations of dark chocolate for both enamel and dentin microhardness. The result shows a significant difference (p-value < 0.05) between each group of concentrations. Notably, the only exception is between concentrations of 70% and 85%, where the p-value of 1 was not significant.

Table 3. A comparison of the enamel microhardness values between pairs of groups.

Group	Artificial Saliva *p*-Values	70% Dark Chocolate	85% Dark Chocolate	100% Dark Chocolate
Artificial saliva	-	0.0001	0.000039	<0.001
70% Dark Chocolate	0.0001	-	1.000	0.000264
85% Dark Chocolate	0.000039	1.000	-	0.001
100% Dark Chocolate	<0.001	0.000264	0.001	-

Table 4. A comparison of the dentine microhardness values between pairs of groups.

Group	Artificial Saliva	70% Dark Chocolate	85% Dark Chocolate	100% Dark Chocolate
Artificial saliva	-	<0.001	<0.001	<0.001
70% Dark Chocolate	<0.001	-	0.000050	<0.001
85% Dark Chocolate	<0.001	0.000050	-	0.00001
100% Dark Chocolate	<0.001	<0.001	0.00001	-

4. Discussion

Enamel and dentin microhardness testing lack a universally established set of conditions, leading researchers to determine testing parameters based on their discretion. Many past studies on microhardness have presented outcomes for both Knoop hardness number (KHN) and Vickers hardness number (VHN) across various indentation loads and durations [19]. Testing under different conditions is motivated by various considerations. Opting for a high load is advantageous as it results in a substantial impression, facilitating the measurement of the indentation diagonal. However, applying a high load to a soft surface can lead to an oversized impression where the diagonals exceed the micrometer scale available in the tester's eyepiece. In the context of a pre-post experimental study, such as investigating a treated surface, it becomes essential to use a smaller load to ensure a meaningful comparison between the baseline and treated surfaces, especially when employing the same indentation load [20]. In studies focusing on tooth hardness, the Vickers indenter is often preferred over the Knoop indenter. This preference stems from the inherent advantages of the Vickers indenter, notably its square shape, which must be consistently preserved. This characteristic is valuable because it ensures uniformity in the indentation geometry. Additionally, the Vickers indenter is advantageous when dealing with nonflat surfaces or variations in hardness between enamel and dentin. Its square indentation shape makes it easier to detect and analyze variations caused by surface irregularities or differences in dental tissue hardness. This makes the Vickers method particularly suitable for dental research, where precise and easily discernible indentations are crucial for accurate hardness measurements [21].

While there is no specific research focusing on the impact of dark chocolate on tooth hardness, the Vickers hardness number (VHN) values acquired for enamel and dentin in this investigation correspond with findings from prior studies that have explored the effects of theobromine, the primary ingredient in cocoa [6,8]. For example, the hardness of enamel has been reported in the range from 236.09 to 243.34 for the control group (artificial saliva) in comparison, G2 (70% dark chocolate), G3 (85% dark chocolate), and G4 (100% dark chocolate) displayed average mean differences spanning from 235.09 to 296.81, 235.53 to 299.70, and 235.95 to 345.10, respectively; and for dentin, the hardness has been reported in the range from 51.31 to 60.95 for the control group (artificial saliva) in contrast, G2 (70% dark chocolate), G3 (85% dark chocolate), and G4 (100% dark chocolate) exhibited average mean differences ranging from 51.79 to 109.70, 51.81 to 124.10, and 51.53 to 140.20, respectively. The observed broad variation in the standard deviation of hardness observed in this study is consistent with findings from previous reports. This consistency in results across various experimental conditions and methodologies underscores the reliability and reproducibility of the study, further supporting the validity of the reported enamel and

dentin hardness values [21]. The wide variation in hardness values can be attributed to factors such as specimen preparation, diagonal length reading [21], variations in chemical composition [22], age, and the specific location within the tooth [23].

As a main canned product, many individuals believe that chocolate is most likely responsible for tooth cavities. In fact, chocolate is comparatively the most cariogenic substance, likely due to its high sugar content, as shown by a variety of studies [24,25]. Actually, cocoa butter found in chocolate is believed to provide a protective layer for the teeth, forming a buttery coating that can fend off damage from the sugar contained in dark chocolate; furthermore, cocoa contains a component that is capable of preventing tooth decay and dental caries [26]. There is evidence that cocoa powder inhibits dental caries in hamsters and that zero-fat cocoa exhibits significant anticary properties [27]. In spite of the fact that the cocoa bean's husk cannot be found, it exists even when the cocoa bean is in its natural state [28]. Another advantage of chocolate manufactured from cocoa beans is that the cocoa bean itself contains anticariogenic substances. The cocoa bean husk, a by-product of the chocolate industry, is renowned for being rich in polyphenols, which can prevent some sugars from turning into acid and breaking down the enamel of the tooth, and nutritional fiber, including cellulose, pectin, and lenin [28]. In the processing of theobroma cacao, the preroasted beans have been extracted from the cocoa bean husk [29]. It has been found that the cocoa bean husk contains two distinct types of cariostatic compounds, with one exhibiting antiglucosyltransferase (GTF) activity while the other demonstrates antibacterial properties [29]. Theobromine is one of the primary components of cocoa beans. Theobromine has a chemical formula of 3.7 dimethylxanthine and is an alkaloid of the cacao plant [30]. It was previously known as xantheose, a crystalline, bitter powder that is insoluble in water. It differs from caffeine by just one methyl group and is thus present in foods such as chocolate, teas, and other beverages. The tea plant's leaves and the kola (or cola) nut both contain it. Through strengthening tooth enamel, theobromine lowers the incidence of tooth decay [30]. Cocoa beans naturally contain 1–4% theobromine. Dark chocolate has higher theobromine contents than milk chocolate, which ranges in cocoa powder from 1.2% to 2.4% [31]. In addition, it contains unsaturated free fatty acids like oleic and linoleic acids, which are antibacterial for Streptococcus mutans [32]. The larger molecular weight of polyphenolic molecules present in cocoa bean husk extract possesses significant antiglycosyltransferase activity. The cariostatic effects of the cocoa bean husk are due to these physiologically active substances [32]. Streptococcus mutans is recognized as the primary contributor to dental caries in humans. It produces three types of glucosyltransferase (GTF), namely GTFB, GTFC, and GTFD. These enzymes are responsible for synthesizing glucans from sucrose, resulting in an adhesive and water-insoluble substance that firmly adheres to the tooth surface [32]. The adhesive glucan, where acid accumulation takes place, plays a role in the formation of dental plaque, leading to localized demineralization of the tooth enamel surface [33]. Previous studies investigating the mechanism of the anticariogenic properties of theobromine have revealed the development of a medium with an apatite structure that promotes the rehardening of the tooth surface through remineralization [34].

In this study, enamel and dentine surface microhardness assessments were conducted utilizing a digital micro-Vickers hardness tester (Laryee, Beijing, China) under (0.24 N load for 15 s) before and after immersion of the specimens in the selected concentration of dark chocolate and using artificial saliva as a control group.

The findings of the current investigation indicated that there is an increase in enamel and dentin hardness after immersion of the prepared specimen in different concentrations of dark chocolate and even in artificial saliva. Therefore, the current study's null hypothesis was rejected. The possible explanation for this effect is attributed to the composition of artificial saliva, specifically the inorganic components, primarily calcium and phosphate. No significant difference was observed between human saliva and artificial saliva containing mucin, suggesting that it can serve as a suitable substitute for human saliva in in vitro cycling studies focused on remineralization and hardness assessment [35]. Also in the major

composition of dark chocolate are minerals like iron, magnesium, zinc, copper, potassium, selenium, phosphorus, and manganese, which are the essential minerals for strengthening tooth structure, according to certain previous studies. Theobromine increased the hardness of the enamel, and the inorganic minerals were directly related to enhancing the hardness of the enamel [4,6]. That agrees with our study. In the present investigation, 70% dark chocolate was employed, characterized by a composition of 70% cocoa along with cocoa butter and sugar. Likewise, 85% dark chocolate was utilized, incorporating 85% cocoa, cocoa butter, and sugar, while 100% dark chocolate consisted solely of cocoa and butter without the addition of sugar. The study revealed a notable correlation between the increasing concentration of dark chocolate and a significant enhancement in the hardness of enamel and dentin, attributable to the heightened cocoa content. This study specifically examined the authentic impact of dark chocolate on enamel and dentin hardness without resorting to demineralization processes, demonstrating a favorable influence on dental structures. Particularly noteworthy is the observed increase in theobromine and mineral content with escalating chocolate concentration. Prior research has primarily focused on the impact of theobromine post-demineralization; however, a dearth of literature exists regarding the direct examination of the inherent effects of dark chocolate on enamel and dentin.

According to the studies, theobromine outperformed fluorides in safeguarding teeth. In a laboratory experiment, researchers evaluated the effectiveness of a ginger, honey, and chocolate mixture in remineralizing the initial enamel caries lesions. The results indicated that there was no noteworthy distinction in surface hardness or mineral loss/gain between the group using bitter chocolate and the group using fluoride toothpaste [36].

Another in vitro investigation compared the antibacterial activity of three distinct toothpastes. First up is theobromine toothpaste, a fluoride-free chocolate-based toothpaste; the other two are two types of toothpaste with fluoride. Results revealed that toothpaste containing theobromine not only displayed a larger zone of microbial inhibitions when compared to other fluoride toothpastes, but also demonstrated enamel remineralization because nontoxic fluoride was absent. As a result, it blended easily with phosphate and calcium to speed up the process of remineralizing the enamel [37]. In addition, individuals with dental fluorosis, tooth discolorations, and hyperplasia of the enamel responded better to theobromine toothpaste [38]. Another study comparing the antibacterial properties of chlorhexidine mouthwash with cacao bean husk extract mouthwash found equivalent substantial decreases in salivary Streptococcus mutans counts in both groups during every follow-up period, demonstrating that this mouthwash is an acceptable substitute for chlorhexidine [39]. Historically, fluoride has been the initial choice in dental practice for preventive purposes [40]. More recently, casein phosphopeptide amorphous calcium phosphate [41] and biomimetic hydroxyapatite [42] have been introduced and have demonstrated encouraging outcomes. In the future, it would be intriguing to assess the synergistic effects of these fluoride-free products when used in conjunction with chocolate-based toothpastes and mouthwashes.

The study has limitations, as it was carried out in a controlled laboratory setting, making it difficult to completely replicate the authentic characteristics of the human mouth. Consequently, the findings may not fully capture the complexities of real-world oral conditions. To enhance the relevance and applicability of the research, it would be advantageous to replicate similar studies in a natural oral environment.

5. Conclusions

Within the limitations of this in vitro study, it can be affirmed that dark chocolate exhibits a protective effect on both enamel and dentine. As the concentration of dark chocolate increases, there is a corresponding increase in the hardness of both enamel and dentine. This enhancement is attributed to the presence of theobromine, alongside numerous minerals and antioxidants found in dark chocolate. Therefore, it can be deduced that incorporating dark chocolate into an individual's diet may benefit in stabilizing the progression of tooth structure loss, offering a potentially tooth-friendly addition to our

diets. However, it is important to recognize that further research, particularly in clinical settings, is needed to confirm and expand upon these findings before definitive dietary recommendations can be made.

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