Original Article

In Vitro Cytotoxic and Remineralization Effects on the Tooth Enamel Surface of Theobromine after the Artificial Induction of Dental Carious Lesions

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Abstract

Background: Theobromine is a methylxanthine alkaloid, and it is mainly found in cocoa and chocolate. It has the ability to enhance the size of hydroxyapatite crystals in enamel, leading to precipitation on the enamel surface and slowing down the breakdown of hydroxyapatite. **Objective:** This experimental study is to evaluate and compare the cytotoxic activity and remineralization effect on dental enamel between theobromine and sodium fluoride following the creation of artificial carious lesions. **Materials and Methods:** The study consisted of two components: In the first segment, theobromine's cytotoxic effects were examined on a human being's ordinary cell line created from human fibroblasts from the skin, using the Mosmann tetrazolium dye assay. The plates were incubated with theobromine at the following concentrations: 50, 100, 200, and 400 mg/L under 5% CO_2 at 37°C. Each concentration, along with the negative control cells without tested material, was tested in triplicate. In the second part of this study, 34 healthy human premolars were randomly allocated to three groups with 11 teeth each. After they had formed artificial carious lesions, group 1 received theobromine, group 2 received sodium fluoride, and group 3 received artificial saliva as the negative control. The treatment solution was delivered dynamically by utilizing a pH-cycling model. Samples were examined through scanning electron microscopy and energy-dispersive X-ray analysis. The data were statistically analyzed. **Results:**There was no significant difference between theobromine's cytotoxicity and the negative control on the normal human cell line ($P > 0.05$). However, the atomic percentages of calcium (Ca) and phosphorus (P) were significantly higher ($P < 0.05$) in theobromine-treated teeth compared to fluoride-treated teeth. **Conclusion:** Due to its toxicity and beneficial remineralization effect, theobromine shows promise as an effective and viable alternative to sodium fluoride.

Keywords: Enamel remineralization, Mosmann's tetrazolium assay, scanning electron microscopy, sodium fluoride, theobromine

Introduction

One of the most prevalent chronic pediatric disorders, dental caries affects children across numerous third-world as well as emerging countries.[1,2] Both primary and secondary variables, including insufficient biofilm clearance, overconsumption of a cariogenic diet, and decreased salivary flow, can be blamed for the early microscopic signs of caries on the enamel surface.[3-6] Whenever the level of mineral depletion from a tooth's subsurface outpaces the rate of mineral intake over a longer period of time, a white spot lesion develops.[4,7-9] Fluoride promotes remineralization by enhancing the resilience of enamel to acid attack through the absorption of ions from oral fluids and total surface absorption on fractionally demineralized crystals, thereby limiting mineral loss from the tooth enamel surface.[10,11] Although fluoride is effective in preventing tooth decay, its excessive use may cause dental and skeletal fluorosis.[12] There is a growing public interest in natural and herbal-based healthcare

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solutions, specifically in the realm of dentifrices. Clinical research has been conducted to validate the efficacy of these products, and biocompatible and natural dental preventive products, such as theobromine, are attracting attention.[13]

Theobromine is a methylxanthine alkaloid, and it is mainly found in cocoa and chocolate. Chocolate contains theobromine at doses that are highly safe for humans to consume in considerable quantities. Previous studies have reported that chocolate has anticaries properties. It shares similar effects with caffeine, a bioactive compound known for its stimulant properties.[13-15]

Theobromine has the ability to enhance the size of hydroxyapatite crystals in enamel, leading to precipitation on the enamel surface and slowing down the breakdown of hydroxyapatite. Additionally, theobromine can be used in the creation of toothpaste tubes to add a pleasant chocolate flavor that appeals to children.[16-18] Furthermore, theobromine has been demonstrated to have neither remineralizing nor cariostatic properties.[19,20] However, its contradictory results imply that its remineralizing potential demands further study. Although many studies have found theobromine to be nontoxic, there is limited research available on the cytocompatibility of cacao extract, particularly at the concentration of 200 mg/L. The primary objective of this research is to examine the effects of theobromine on cell viability and its possible remineralization effect.

Materials and Methods

Test on the cytotoxicity of cacao extract

Cell line culture and maintenance

In Roswell Park Memorial Institute (RPMI) media, a human ordinary cell line originating from neonatal human skin fibroblasts (HDFn) was grown and kept alive using methods for cell line culturing, specifically Mosmann's tetrazolium toxicity (MTT).[21] After a confluent monolayer of cells had formed in the flask, the growth medium was removed and the cell sheet was subsequently washed with phosphate-buffered saline. The cells were then treated with 2–3 mL of trypsin solution and incubated at 37°C for 1 min, resulting in their detachment from the container and their subsequent isolation as individual entities rather than as a monolayer. The medium was then replaced with new, comprehensive RPMI media. The cells were then dispensed into culture tubes at the proper concentration. and incubated for 24 h at 37°C and 5% CO_2.

Cell viability assay

The most widespread, quick, and simple approach was used to assess the *in vitro* cytotoxicity of cacao extract. MTT assay uses a yellow dye that is converted into purple formazan; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, which is converted into purple formazine by the mitochondrial enzyme succinate dehydrogenase, an enzyme present only in healthy, viable cells, lyses the tetrazolium ring.[21] A complete culture medium was used to cultivate normal cells in 96-well microplates at a concentration of 1×10^4–1×10^6 cells/mL. Each plate was then kept in a 37°C CO_2 incubator for 24 h. After 24 h, the medium was disposed of, and the wells were refilled with theobromine solutions of varying concentrations (50, 100, 200, and 400 mg/L). The dishes were then left to incubate for an additional day at 37°C with 5% CO_2. Cells without the extract were also cultured on the same plates to serve as the negative control. A total of 10 µL of MTT solution was added to each well, and the plates were incubated for an additional 4 h at 5% CO_2. The medium was removed, 100 µL of solubilization solution was added to each well to dissolve formazan crystals. The IC_{50} value, which is the concentration of normal cells at which 50% inhibition occurs, was reported and used to compare the cytotoxic impact of the tested agent with that of the negative control. Absorbance was read at 570 nm with an ELISA reader.[21,22] Triplicates of each concentration, including the negative controls, were prepared. The effect was determined using the following equation:

$$\text{Viability (\%)} = \text{Optical density of the sample} \times 100\%/\text{optical density of the control.}$$

Remineralization effect of theobromine

Sample size

The sample size was calculated in accordance with the data reported from previous studies.[9,23] A sample size of 30 teeth was deemed essential (10 samples per group). An additional four teeth were used to analyze enamel surface morphology using scanning electron microscopy (SEM). A total of 34 premolars indicated for extraction for orthodontic purposes were obtained from patients aged 11–14 years who attended dental clinics in Basra City, Iraq.[24]

Specimen preparation

The selected teeth were stored in a 0.1% thymol solution (M Dent, Bangkok, Thailand) at room temperature after being washed with deionized water. The tooth samples were polished with non-fluoridated pumice.[25] Then, the residual roots were trimmed to a length of 2 mm below the cementoenamel junction using a straight diamond bur with copious irrigation. The plastic cylinder tube (Zhejiang Liutong Plastics Co., Ltd., China) was transformed into rings with a diameter of 20 mm and a depth of 8 mm. These rings were created by cutting the tube into equal sections that were parallel to each other. The resulting rings have flat tops and bottoms. The tooth samples were individually positioned and secured in the ring with the buccal surface facing upward. Subsequently, cold-cure

acrylic resin was carefully poured into each ring, filling it completely (Akrodent, Ankara, Turkey). After placing a strip of tape measuring 4 mm × 2 mm onto the middle third of the crown, the surface was then coated with nail varnish. Once the varnish had dried, the tape was carefully removed, unveiling the underlying enamel.[4,24]

Treatment procedure

Formation of artificial carious lesions

All 34 teeth, except one (which was left normal for later SEM examination), were individually subjected to demineralizing solution (pH 5.4)[17,26,27] for 7 days at a temperature of 37°C. After the treatment period, the teeth were gently rinsed with distilled water for 20 s. The samples were washed with distilled water, dried, and then retested for SEM-energy-dispersive X-ray (EDX) analysis.[26,27]

Treatment solution groups

An individual number ranging from 1 to 33 was assigned to each tooth of the study sample. Following the assignation of numbers, the study samples were allocated randomly into three groups (11 teeth to each group: 10 for SEM–EDX and one for SEM examination).

Group 1: This group was treated with 200 mg/L theobromine-infused artificial saliva (Sigma-Aldrich, Hamburg, Germany).[17,28]

Group 2: This group was treated with sodium fluoride solution (0.05% sodium fluoride powder dissolved in artificial saliva).[17,28]

Group 3: This group was treated with artificial saliva and considered the negative control group.

After being treated with one of the above solutions, each tooth was kept in 10 mL of deionized water in a container labeled with the number and name of the group.

pH cycles

Following the development of a white spot lesion, treatment solutions were administered on a 28-day pH cycle.[17] This cycle involved a demineralization phase lasting 1 h that was repeated three times daily. After the previous phase, a remineralization period of 21 h. in artificial saliva followed. This cyclic process aimed to simulate the conditions of the oral environment. Once the demineralization phase was completed, the treatment solutions were applied for a duration of 2 min. According to Amaechi *et al*.[17], the artificial saliva and demineralization solution were made. Prior to treatment, the pH of the demineralizing solution and artificial saliva was monitored once a day. After being exposed to either the demineralizing or remineralizing solution, the samples were washed with flowing deionized water. They were dried before being dipped into the succeeding solution. The pH cycle for a single day was repeated continuously for 28 consecutive days.[17]

Scanning electron microscopy and EDX

SEM was utilized to examine the changes in the enamel morphology of four teeth: one sound tooth and three others (one sample from each group). To secure the teeth onto the SEM holder, releasable glue was utilized. The teeth were positioned in a manner that allowed their treated surfaces to be prominently visible. At the University of Basra's Physics Department in the College of Science, a sputter coater (Leice EM ce 600 High Vacuum Sputter Coater) was employed to apply a layer of gold and palladium onto each tooth. This process was carried out within a vacuum environment. Subsequently, the treated teeth were placed in a scanning electron microscope (Nova Nano SEM 450) for examination. The SEM operated at an accelerating voltage of 8 kV. Exemplary samples were prepared for SEM analysis at magnifications of 5000× and 10,000×. Two specialists analyzed the surface morphology of enamel in each image to ascertain whether any discernible differences in enamel texture could be observed following the application of different treatment modalities.[4,23] The use of EDX enabled the determination of the weight percentages (wt%) of Ca and P as part of an elemental analytical inquiry or chemical characterization of a substance. EDX depends on the interactions between an X-ray excitation source and the sample being studied. The underlying principle of spectroscopy is based on the fundamental assumption that every element possesses a distinct atomic structure, resulting in a unique arrangement of peaks on its electromagnetic emission spectrum. This characteristic attribute plays a significant role in enabling EDX analysis to effectively characterize various materials.[29]

Statistical analysis

Statistical Package for Social Science (SPSS version 22, Chicago, Illinois, USA) was used for data interpretation, processing, and representation. Experimental measurement data were tested with Shapiro–Wilk's statistics to determine their distributional shape. Given that the data followed a normal distribution, one- and two-way analysis of variance and Tukey's honestly significant difference (HSD) were used to determine if the groups differed statistically. A P value below 0.05 was considered statistically significant.

Ethical approval

The Ethical Committee of the College of Dentistry, University of Baghdad, assessed and authorized the protocol of the current straightforward randomized controlled *in vitro* study on April 17, 2022 (Ref. 549322), and determined that it complied with the Helsinki Declaration and its principles of guidance.[30]

Results

Cytotoxic effect

To assess cell vitality, the formation of formazan crystals linked to MTT decline by live cells was examined. The

Table 1: Mosmann's tetrazolium toxicity assay results of the cell viability of the groups treated with cocoa extract at different concentrations

Groups	Minimum	Maximum	Mean	±SD	±SE	*F*	*P* value
Control	95.01	95.09	95.0450	02,153	00,622	1.424	0.183^{NS}
50	95.02	95.60	95.3350	16,340	04,717		
100	94.01	94.90	94.4442	28,334	08,179		
200	94.03	94.60	94.0933	16,019	04,624		
400	93.30	93.99	93.7758	28,830	08,323		

SD: standard deviation, SE: standard error, NS: non-significant difference between groups (*P* value > 0.05)

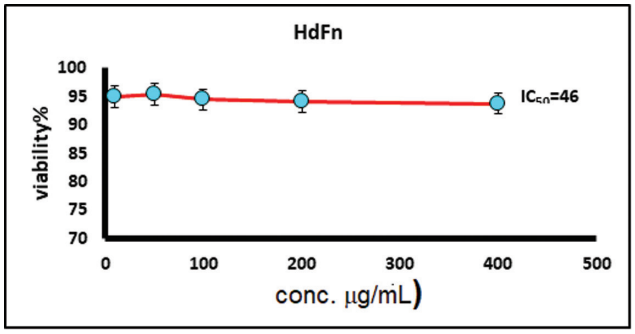

Figure 1: Half-maximal inhibitory concentration of cells on human dermal fibroblast of neonate cell line of cacao extract

MTT test was carried out to evaluate the viability of HDFn in response to theobromine extracts at various concentrations and negative control. The assay provided data on the dose-response relationship, enabling the determination of the IC_{50} (the concentration required to inhibit 50% of cell growth). The results of this analysis are shown in Table 1. The data are expressed as means and standard deviations. Table 1 demonstrates the evaluation of the impact of cocoa extract, containing theobromine, on the vitality of tooth cells using the MTT test at various concentrations. The outcomes are presented as a range of percentages representing the minimum and maximum values of cell viability. These results indicated that the cocoa extract had no observable effect on the living cells. Additionally, theobromine exhibited no toxic effects on the cells at concentrations of 50, 100, 200, and 400 µg/L as indicated by the IC_{50} values. The IC_{50} value was determined to be 46.0 µg/L at a concentration of 400 µg/L [Figure 1]. Again, treatment with theobromine at all concentrations, including the control, resulted in a notably high cell viability. In conclusion, the MTT assay demonstrated that there were no significant differences observed between the IC_{50} values of theobromine at various concentrations and those of the negative control [Figure 2].

Figure 2 illustrates the significance of the differences among the mean cell viabilities of the tested materials at different concentrations and the negative control, which showed no significant differences in HDFn cells between groups at all concentrations when compared with the negative control.

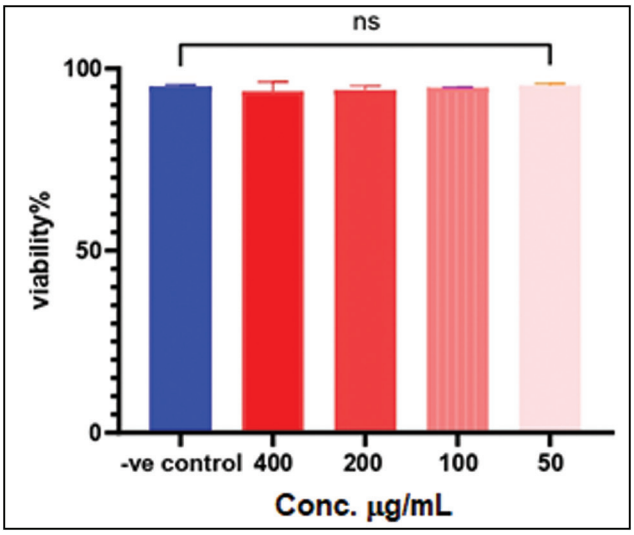

Figure 2: Graph displaying the mean cell viability (%) for the theobromine and negative control groups

Reminerlization

The results of Shapiro–Wilk's test showed that the atomic percentages of Ca and P were normally distributed among groups ($P > 0.05$). Tables 2 and 3 display the mean and the standard deviation of the atomic percentages of Ca and P in the tested groups across three time points (baseline, after demineralization, and after treatment). Following exposure to the demineralization solution, a decrease in the atomic percentages of Ca and P was observed in all study groups. However, after treatment, an increase in Ca and P content was witnessed. Among the treatment groups, the group treated with theobromine and artificial saliva exhibited the highest mean surface levels of Ca and P, whereas the control group showed the lowest mean values. Statistical analysis revealed a significant difference among all three time points in each of the three groups.

Tukey's HSD was employed to conduct multiple pairwise comparisons of the atomic percentages of calcium (Ca) and phosphorus (P) among the groups in various phases. The results of these comparisons are presented in Tables 4 and 5. No significant differences were observed in the theobromine group between the baseline phase and the treatment phase. Similarly, Table 5 shows no significant differences in the control group.

Table 2: Descriptive statistics and repeated measures analysis of variance (ANOVA) test for calcium (Ca) levels across different stages and groups

Groups	Baseline		Demin.		Treat.		*F*	*P* value	Effect size
	Mean	±SD	Mean	±SD	Mean	±SD			
Theobromine + saliva	26.197	0.868	16.014	1.184	26.061	1.318	1091.734	0.000	0.987
Sodium fluoride + saliva	25.780	0.941	13.334	.953	17.337	1.151	401.421	0.000	0.966
Control	24.872	1.594	9.367	.695	10.341	.529	520.440	0.000	0.973
F	2.115		80.717		373.286				
P value	0.095		0.000		0.000				
Effect size	0.158		0.878		0.971				

*ANOVA was used. *Significant at $P < 0.05$

Table 3: Descriptive statistics and repeated measures analysis of variance test for phosphorus (P) levels across different stages and groups

Groups	Baseline		Demin.		Treat.		*F*	*P* value	Effect size
	Mean	±SD	Mean	±SD	Mean	±SD			
Theobromine + saliva	16.266	.515	11.200	.353	12.741	.385	514.297	0.000	0.973
Sodium fluoride + saliva	15.605	.927	9.028	1.132	9.900	1.349	607.568	0.000	0.977
Control	15.233	.832	7.320	.569	7.562	.533	750.952	0.000	0.981
F	2.290		36.464		49.996				
P value	0.074		0.000		0.000				
Effect size	0.169		0.764		0.816				

*Significant at $P < 0.05$

Table 4: Multiple pairwise comparisons of calcium (Ca) levels across stages within groups were conducted using Tukey's honestly significant difference

Groups	(I) Stages	(J) Stages			
		Demin.		Treat.	
		Mean difference	*P* value	Mean difference	*P* value
Theobromine + saliva	Baseline	10.183	0.000	.136	1.000
	Demin.			−10.047	0.000
Sodium fluoride + saliva	Baseline	12.446	0.000	8.443	0.000
	Demin.			−4.003	0.000
Control	Baseline	15.505	0.000	14.531	0.000
	Demin.			−.974	0.000

*Significant at $P < 0.0$

Table 5: Multiple pairwise comparisons of phosphorus (P) levels across stages within groups were conducted using Tukey's honestly significant difference

Groups	(I) Stages	(J) Stages			
		Demin.		Treat.	
		Mean difference	*P* value	Mean difference	*P* value
Theobromine + saliva	Baseline	5.066	.000	3.525	.000
	Demin.			−1.541	.000
Sodium fluoride + saliva	Baseline	6.577	.000	5.705	.000
	Demin.			−.872	.000
Control	Baseline	7.913	.000	7.671	.000
	Demin.			−.242	.095

*Significant at $P < 0.0$

Tables 6 and 7 present noteworthy differences between the control group and the theobromine group, which was treated with artificial saliva, during both the treatment and demineralization stages. Additionally, a notable difference was observed between the control group and the group treated with sodium fluoride. However, there was

Table 6: Multiple pairwise comparisons of calcium (Ca) levels among groups were conducted using Tukey's honestly significant difference

Stages	(I) groups	(J) groups	Mean difference	*P* value
Demin.	Control	Theobromine + saliva	−6.647	0.000
		Sodium fluoride + saliva	−3.967	0.000
	Theobromine + saliva	Sodium fluoride + saliva	2.680	0.000
Treat.	Control	Theobromine + saliva	−15.720	0.000
		Sodium fluoride + saliva	−6.996	0.000
	Theobromine + saliva	Sodium fluoride + saliva	8.724	0.000

*Using Tukey's honestly significant difference. *Significant at $P < 0.05$

Table 7: Multiple pairwise comparisons of phosphorus (P) levels among groups were conducted using Tukey's honestly significant difference

Stages	(I) groups	(J) groups	Mean difference (I– J)	*P* value
Demin.	Control	Theobromine + saliva	−3.880	0.000
		Sodium fluoride + saliva	−1.708	0.000
	Theobromine + saliva	Sodium fluoride + saliva	2.172	0.000
Treat.	Control	Theobromine + saliva	−5.179	0.000
		Sodium fluoride + saliva	−2.338	0.000
	Theobromine + saliva	Sodium fluoride + saliva	2.841	0.000

*Using Tukey's honestly significant difference. *Significant at $P < 0.05$

no significant difference found between the theobromine group and the sodium fluoride group ($P > 0.05$).

Surface morphology evaluation using scanning electron microscopy

1. Sound tooth surface: SEM analysis revealed that the sound enamel surface had a smooth and homogeneous pattern and keyhole or paddle-shaped prisms [Figure 3a].
2. Control: SEM images illustrated the microscopic modification of the enamel surface treated with artificial saliva, revealing the lack of typical enamel perikymata, fissures, depressions, the formation of tiny holes, and unevenly melted and recrystallized regions. Additionally, enamel acid dilution resulted in the exposure of prisms as presented in Figure 3b.
3. Theobromine-treated tooth: SEM showed surface changes in the enamel treated with artificial saliva. The deposition of particles on the demineralized enamel surface was smooth and uniform, nearly closing the microspores created after demineralization. The deposited particles were distributed across the enamel surface, filling surface defects and reducing the roughness caused by demineralization. Partial fusion and recrystallization of enamel prisms can also be observed in Figure 3c
4. Sodium fluoride-treated tooth: Morphological alterations in the teeth treated with sodium fluoride were observed as amorphous, globular, and crystalline formations obstructing the microspores generated after demineralization. At the same time, the surface maintained its rough texture as shown in Figure 3d.

Discussion

The present study investigated the cytocompatibility of theobromine using human cell lines. The MTT assay is widely utilized to determine the cytotoxic effect of diverse substances across a range of conditions or concentrations.[31-33] The HDFn cell line was selected due to its properties being comparable to those of human gingival fibroblasts. In addition, the IC_{50} value (50% of the half-maximal inhibitory concentration) of relevant medications can be determined through comparison with the survivability of the negative control group.[21] In this study, no significant differences were observed between the low concentrations of theobromine, a cocoa extract, and the negative control. Furthermore, theobromine did not exhibit any detrimental effects on the viability of normal HDFn cells. These findings indicated that theobromine is nontoxic or has minimal toxicity towards HDFn cells, particularly at very high concentrations. Consequently, theobromine can be considered a safe alternative to sodium fluoride as a dental suspension substitute for the prevention and treatment of dental caries. Consistent with previous studies, these findings support the notion that theobromine is highly safe for human consumption, even at high concentrations. Theobromine, derived from cacao, is a natural substance and is currently recognized as a dietary supplement for individuals. Despite decades of popular use of chocolate treats, there have never been any reported cases of theobromine poisoning in humans.[34,35] The results of the present study contradict earlier findings, which suggested that theobromine could have negative effects. It has been observed that certain animal species, such as dogs and horses, are more

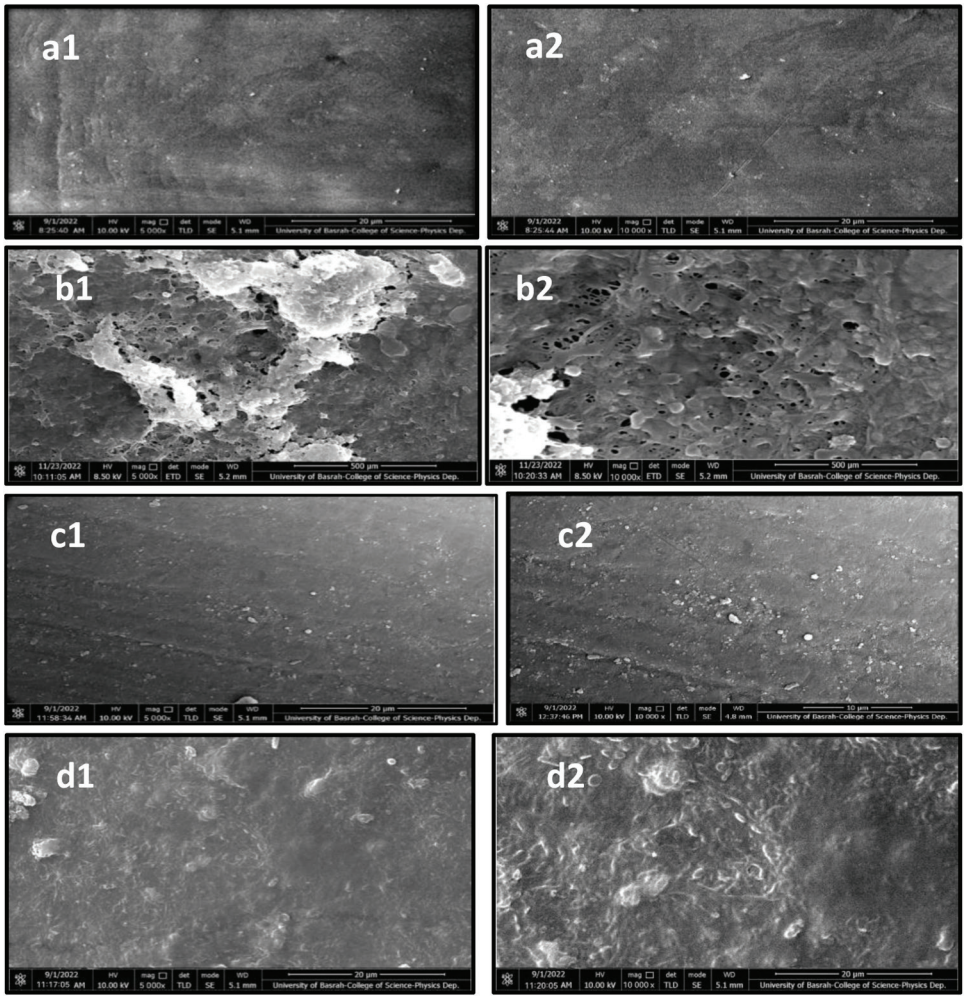

Figure 3: (a) Scanning electron microscopy (SEM) image of a sound tooth (a1. 5000×, a2. 10,000×). (b) SEM image of the control group (b1. 5000×, b2. 10,000×). (c) SEM image of the theobromine group (c1. 5000×, c2. 10,000×). (d) SEM image of the sodium fluoride group (d1. 5000×, d2. 10,000×)

susceptible to the negative effects of methylxanthine (theobromine) compared to others. The variation is likely due to differences in how theobromine is metabolized in humans compared to animals.

The aim of the present study was to compare the remineralizing effects of theobromine and sodium fluoride on white spot lesions. A 0.05% concentration of sodium fluoride solution, suitable for young children, was utilized. Theobromine, on the other hand, was tested at a concentration of 200 mg/L, which demonstrated significant potential for remineralization according to a previous study.[28] To induce subsurface lesions, a demineralizing solution with a pH of 5.4 was employed.[26,27] Artificial saliva serves a twofold purpose in dental research. Firstly, it mimics the natural composition of saliva, closely resembling its chemical makeup. Secondly, it transforms the oral cavity into a simulated environment that effectively prevents bacterial contamination of enamel specimens.[17] The demineralization and remineralization efficacies of the experimental materials were inspected using SEM–EDX, which reliably detects ion levels at very low concentrations within highly mineralized tissue.[16,36]

Demineralization, which is a precursor to carious lesions (white spot lesions), was associated with a significant decrease in the atomic percentage of Ca and P in all groups. Following demineralization, a significant decrease in the atomic percentage of Ca and P on the enamel surface was observed in all groups after 7 days in the demineralization solution. The reduction in the percentage of Ca and P was attributed to the acidic environment created when the pH of the surrounding environment fell below the critical pH of 5.5. The acidic medium facilitates the leaching of minerals, primarily Ca and P, from the tooth structure. This process leads to the formation of microspores and a subsequent decrease in the levels of Ca and P. During the treatment stage, the theobromine and sodium fluoride groups demonstrated greater remineralization potential than the control group. However, a statistically significant difference was observed between the groups, with the mean atomic percentage of Ca and P in the

theobromine group being higher than that in the sodium fluoride group. The findings of this study are consistent with previous research,[16,35,36] while several earlier studies either overlooked the remineralization potential of theobromine or considered it to be less effective compared to fluoride.[19,37,38] These discrepancies could be attributed to variations in the composition of the demineralization and remineralization agents utilized in different studies. Furthermore, in the current study, a pH-cycling model was employed instead of continuous acid exposure, which was utilized in previous investigations. The SEM photos from this study's research showed that the enamel surface treated with theobromine seemed smooth and similar to the intact enamel surface before demineralization, which is consistent with earlier findings.[14,39] Similar to earlier studies, the fluoridated tooth surfaces in this study's SEM pictures showed a tough surface roughness with little pores.[40]

The study's potential limitations included an incomplete simulation of the biological aspects of caries lesions in the *in vitro* model. Further *in vitro* studies involving samples from different populations and the measurement of the atomic percentage of Ca and P at different sites on the tooth enamel surface are needed. Human clinical studies are required to exploit theobromine's benefits for maintaining oral hygiene and preventing dental caries.

Conclusion

In conclusion, theobromine, a natural and safe substance, offers remarkable remineralizing properties. It can serve as a promising alternative to fluoride as a cariostatic agent. When applied to tooth enamel, theobromine has been observed to improve the enamel's appearance, leaving it gleaming and silky smooth.

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Conflicts of interest

There are no conflicts of interest.

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