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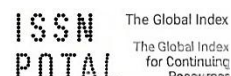
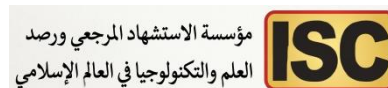
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Influence of Boswellic Acid on Enamel Microhardness in Initial Caries Lesions: An in Vitro Study

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Abstract

Tooth decay is an extremely common condition that needs a preventative mechanism. Acetyl-11-keto- β -Boswellic acid (AKBA) is a natural product derived from *Boswellia serrata* with numerous beneficial properties and its impact on enamel remineralization has not been investigated. This research examined how AKBA affects the microhardness and surface morphology of the enamel. For this investigation, premolars that had been extracted for orthodontic purposes were cleaned, wrapped individually, and stored in distilled water until testing. Each sample went through a total of three testing procedures. The first baseline procedure was to measure the microhardness of the enamel, which had yet to be demineralized. The second demineralization procedure involved demineralization by simulating early lesions

of the enamel in an acidic solution (pH. 4.5). After AKBA was applied, the samples were remineralized and placed into containers with artificial saliva to recreate the conditions of the oral cavity. The best results amongst the test concentrations were with 0.1 μ M AKBA. This indicated a more homogeneous surface that exhibited the greatest microhardness and sufficient mineral content ($p < 0.05$) as confirmed by FESEM and EDX analyses. Overall, amongst the other test concentrations, 0.1 μ M AKBA was the best in increasing the hardness and remineralization of the enamel as well as improving the enamel morphology. Based on the evidence presented, AKBA has the properties of an adjunctive agent for enamel repair and the prevention of demineralization and enhancing microhardness as well as modifying the surface morphology of enamel.

Keywords: Boswellic acid, Enamel microhardness, Initial caries lesions, Remineralization, Frankincense.

Introduction

The enamel consists of 96–97 wt.% inorganic HA ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), along with 3 wt.% water and 1 wt.% organic material (Fejerskov et al., 2015).

The body's most resistant calcified matrix. The cells that produce enamel, the ameloblasts, degenerate upon eruption of the tooth into the oral cavity (Nanci, 2003), and thus, it is hard for enamel to regenerate itself because of the insufficiency of calcium and phosphate ions in saliva (Lacruz et al., 2017). Due to its high physicochemical reactivity and ionic exchange, enamel is a dynamic tissue (Cochrane et al., 2010).

Because of its high mineral content, enamel is the body's most resistant tissue, providing strong resistance to mechanical stress and wear. This is crucial because enamel cannot regenerate once lost (Fehrenbach & Popowics, 2020). A strong bond at the dentinoenamel junction (DEJ) is essential to resist masticatory forces. Enamel thickness is highest at the incisal and occlusal surfaces and gradually decreases toward the cementoenamel junction (CEJ) (Boushell & Sturdevant, 2022). Dental enamel consists mostly of hydroxyapatite (90–92%), with minor amounts of water, trace minerals, and organic matrix (Ritter, 2025).

Recently, increasing attention has been directed toward bioactive natural extracts as safer and effective alternatives or additions to synthetic drugs. Frankincense is among the most extensively used medicinal plants due to its different biological activities (Abbood et al., 2022). Traditional medicine has long used natural plants to treat several diseases. Frankincense (olibanum) is recognized for its beneficial pharmacological effects and low toxicity, its name is derived from the old French word for frankincense, which signifies pure incense, whereas “olibanum” traces back to both Arabic and Latin origins (El-Nagerabi et al., 2013; Van Vuuren et al., 2010).

The final product consists of about 30-60% resin, 30-55% polysaccharides, and 5-10% essential and aromatic oils (Siddiqui, 2011). For centuries prior to their adoption in European cultural and religious ceremonies, the resins from these *Boswellia* species were used in traditional medicine across India, China, and the Arabian region. For twenty years, extensive studies have highlighted frankincense's potential as a therapy. Efferth and Oesch conducted a systematic review of *Boswellia* species' anti-inflammatory and anticancer effects, covering bioactive compounds such as 3-O-acetyl-11-keto- β -Boswellic acid (AKBA) and other Boswellic acids (Efferth & Oesch, 2022). Frankincense resin contains abundant triterpenoid Boswellic acids, comprising over twelve known compounds. The most biologically relevant of these are 11-keto- β -Boswellic acid (KBA), 3-O-acetyl-11-keto- β -Boswellic acid (AKBA), and α - and β -Boswellic acids (Al-Yasiry & Kiczorowska, 2016; WHO, 2009).

Aldandan et al. 2024 showed that Acetyl-11-keto- β -Boswellic acid (AKBA) promotes osteoblast differentiation and bone regeneration, the present study investigated its effect on demineralized enamel by evaluating changes in enamel microhardness as an indicator of remineralization potential (Aldandan et al., 2024). Boswellic acid-based extracts show favorable safety, biocompatibility, and soft-tissue compatibility, supporting their use as natural alternatives to conventional dental chemical agents (Salman et al., 2021). Such evidence supports their use in the current study as a safe, biologically active natural source with a proven effect.

Materials and Methods

Sample preparation

fifteen specimens were used to identify the optimal AKBA concentration with G*Power version 3.1.9.7. setting power at 95%, alpha error probability of 0.05, and a large effect size ($f = 0.4$), as

calculated using As authorized by the health research committee (Ref No. 970, 18/11/2024), fifteen sound human permanent maxillary first premolar teeth which had comparable dimensions. The patients' age ranged from 12-20 years. The teeth were extracted as a part of orthodontic treatment plan. Subsequently, the teeth underwent a one-week immersion in 0.1% Thymol solution, an antimicrobial agent employed to inhibit bacterial growth. They were thereafter stored in distilled water until required. All tooth roots were sectioned at the cemento-enamel junction, perpendicular to the tooth's long axis. This was accomplished with a diamond cutting disc fitted to a cutting machine including water cooling. (Isomet 1000, Buehler, Lake Bluff, IL, USA) at CEJ consuming a water-cooled diamond blade (330-CA/RS-70300, Struers, Detroit Rd. Westlake, LLC, Cleveland, OH, USA). Each crown was split into two halves, and only one of the halves, the buccal half, was saved. Each surface was cut into 3 slabs as 4.0 mm (length) $\times$ 4.0 mm (width) $\times$ 2.0 mm (thickness), and these slabs were embedded in epoxy resin molds. All specimens were polished (Laryee Technology CO.LTD, China) under water-cooled (silicon carbide) using a sequential pattern of P1200 for 10 s, P2500 for 10 s, and P4000 for 4 min to achieve smooth surfaces (Al-Shareefi et al., 2022; Shubbar et al., 2023) and then ultrasonically cleaned for 4 min to remove smear layer. Afterwards, these slabs were subjected to pH-cycling (4.5-7) for 10 days immersed in 25 mL of demineralization solution for 3 hours, followed by 21 hours in artificial saliva, The specimens were incubated at 37°C.

(Ten Cate & Duijsters, 1982).

A 10-day remineralization pH cycling model was used to replicate oral pH dynamics, comprising 3 hours of demineralization and 21 hours of remineralization (151). For three days, the pH cycles continued with no treatment applied (155). Subsequently, over the following days (after day 7), the treatment was administered once daily after the demineralization period, for a duration specific to each agent. Teeth from all groups were individually immersed in the demineralization solution for three hours, exposed to AKBA (2-4 mins), rinsed thoroughly, dried, and then kept in artificial saliva for twenty-one hours.

Artificial saliva preparation

The artificial saliva solution was prepared according to Amaechi and Higham 2001. The artificial saliva solution consists of:

1. MgCl₂.6H₂O (0.148 mmol/l).
2. K₂HPO₄ (4.59 mmol/l).
3. Dihydrogen Potassium Phosphate (KH₂PO₄), KCl, (8.39 mmol/l).
4. Calcium lactate (1.76 mmol/l), fluoride (0.05 ppm)
5. Sodium Carboxymethyl Cellulose (2.25 mmol/l).
6. methyl-4-hydroxybenzoate (13.14 mmol/l).

During the experimental time, no precipitation was seen in the solution when the pH was changed to 7.2 using KOH (Potassium hydroxide). Artificial saliva is made to resemble real saliva in texture and color by adding sodium carboxymethyl cellulose (CMC) to make it thicker and preservative-free.

Preparation of the AKBA solutions

Boswellic acid was selected for its potential remineralizing and anti-inflammatory properties. The material was taken in powder form and centrifuged to accomplish uniform particle distribution, allowing fine particles to settle and products a homogeneous powder proper for precise solution preparation.

Acetyl-11-keto- β -Boswellic acid (AKBA) powder (Cat. No. HY-N0892R, CAS No. 67416-61-9, MW 512.72 g/mol) was purchased from MedChemExpress (MCE, USA). The compound was provided as a research-grade material (for research use only) and stored at 4°C in a sealed container, protected from moisture and light. A stock solution was then prepared by precisely weighing the required amount of AKBA powder using an analytical balance (KERN ALS 220-4N, KERN & Sohn GmbH, Balingen, Germany) and deionized water to get a final concentration of 2 mM. The weighed powder was dissolved in 5 mL of deionized water, and the mixture was completely stirred until a clear and homogeneous solution was achieved. From this stock solution, three working concentrations were prepared: 1 μ M, 0.1 μ M, and 0.01 μ M. These dilutions were made using a micropipette and deionized water via serial dilution. All solutions were kept in clean and sealed containers after being freshly prepared, labeled correctly, and used according to the progress of work. All protocols were conducted under aseptic conditions at room temperature to maintain the accuracy and repeatability of the investigation (Aldandan et al., 2024).

A significant increase in enamel microhardness after demineralization via various treatment techniques can be observed, suggesting that these may play a role in improving enamel resilience (Al_Bazaz et al., 2023).

The microhardness enhancement was related to the morphology of the surface: less and non-homogeneous coating at 0.01 μ M and excessive agglomerates formed at 1 μ M were both characterized by the weakest results, as opposed to a uniform distribution at concentration 0.1 μ M, which strengthened enamel to the greatest extent.

This increased hardness at 0.1 μ M might have been because it could produce a more protective and well-integrated surface layer that functioned as remineralization by increasing surface continuity, which improved resistance to mechanical stress. A uniform and adherent coating can resist enamel from erosion and enhance the solidification of the outer wall. However, 1 μ M did not produce a good interface due to over-compacted and disorganized layer coverage, while the amounts of material for mechanical protection (0.01 μ M) proved inadequate in such a concentration. It thus tells that the optimally distributed layer could assist the enamel surface and increasing its hardness by 0.1 μ M.

The FESEM analysis demonstrated that in comparison to untreated samples, the surface of the AKBA 1 μ M treated enamel was characterized by increased surface roughness attributable to the uneven deposition of a coating that altered the performance of the microhardness by preventing the formation of a stable, well-adhered, and reinforcing layer as shown in Figure 1 (c).

The enamel that was coated with 0.1 μ M AKBA showed the most uniform and homogeneous surface coating among the groups. FESEM images depicted a uniform and continuous layer with low particle aggregation and preserved surface integrity of the enamel. This morphology was paired with the highest microhardness values. This demonstrated that 0.1 μ M AKBA most effectively reinforced the surface of enamel and was the optimal concentration for improving the strength of enamel as shown in Figure 1 (d).

The enamel surface coated with 0.01 μ M AKBA only showed a thin and discontinuous coating. This coating, in comparison to the others, had a poor conforming surface coverage under FESEM; many scattered particles and a poorly coated surface were present. Despite the lack of aggregation, the small quantity of deposited substance was insufficient to exert a protective and strengthening influence on the enamel. Therefore, this group displayed lower microhardness

values, also indicating that the low concentration was not sufficient to improve the enamel surface or mechanical properties as shown in Figure 1 (e).

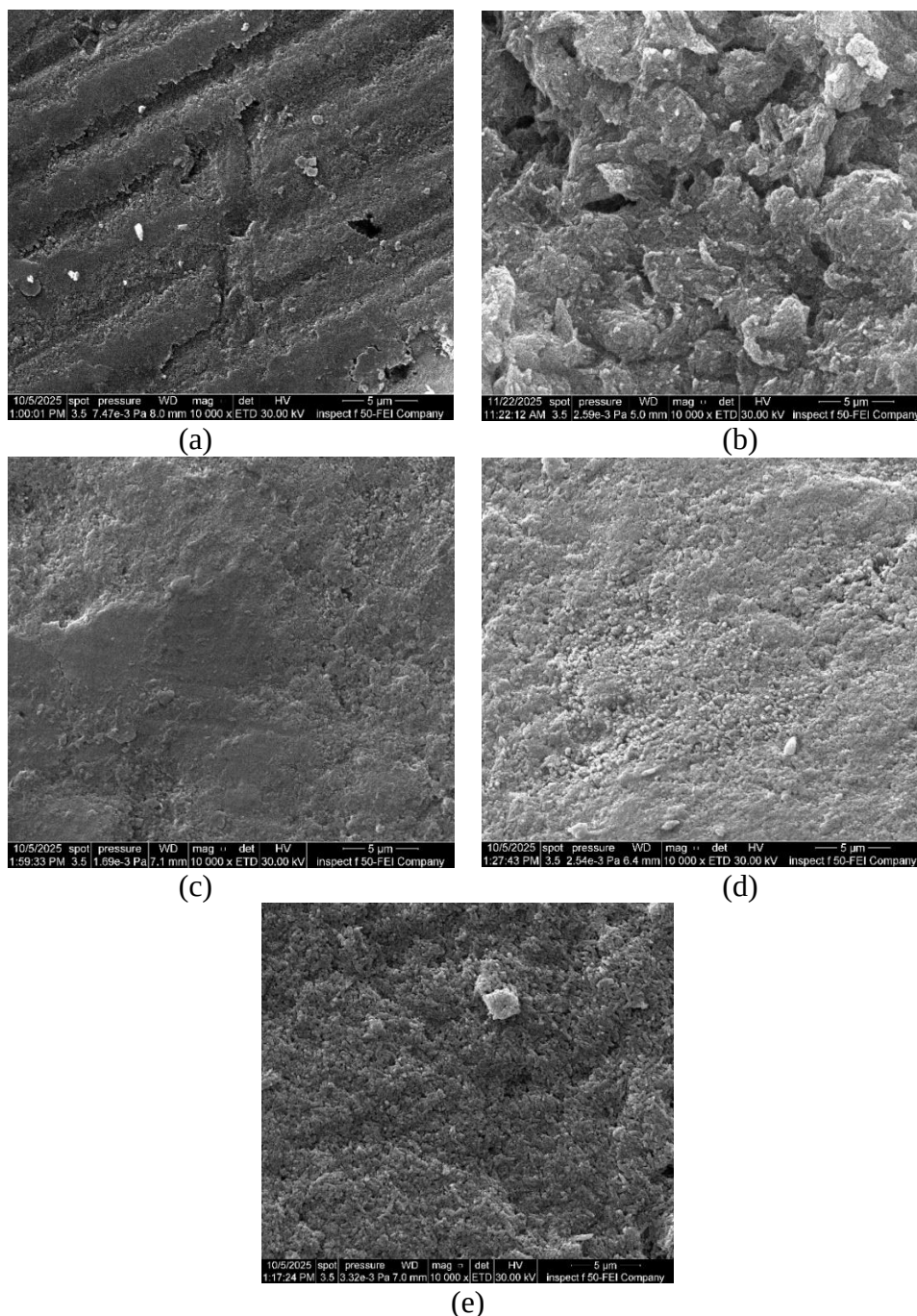


Figure 1. FESEM images illustrating enamel surface morphology at different experimental stages, (a) baseline enamel exhibiting an intact and smooth surface with well-defined enamel prisms, (b) demineralized enamel showing surface irregularities, increased porosity, and loss of prism integrity, (c) FESEM micrographs of enamel surfaces treated with AKBA at 1 μM showing dense aggregation and irregular morphology, (d) FESEM micrographs of enamel

surfaces treated with AKBA at 0.1 μM demonstrating a uniform and continuous layer with minimal aggregation, (e) micrographs of enamel surfaces treated with AKBA at 0.01 μM showing discontinuous surface coverage with scattered particles.

In Energy-dispersive X-ray spectrometry (EDX), paired sample comparisons between demineralization and treatment also confirmed this effect. In the 0.1 μM group, phosphorous and calcium both demonstrated significant increases after treatment ($p = 0.031$ for phosphorous, $p = 0.041$ for calcium), implying effective remineralization. In the 1 μM and the 0.01 μM groups, there was an improvement tendency; however, these changes were not statistically significant as shown in Table 1.

Table 1. Paired sample statistics from demineralization to treatment phases in every group.

Groups		Paired Differences (Mean)	t	p value
1	Dem. P - treat. P	-3.200	-3.990	0.057
	Dem. Ca - Treat. ca	-8.100	-3.967	0.058
0.1	Dem. P - treat. P	-4.033	-5.587	0.031
	Dem. Ca - Treat. ca	-8.533	-4.764	0.041
0.01	Dem. P - treat. P	-3.233	-5.332	0.033
	Dem. Ca - Treat. ca	-8.233	-4.251	0.051

These mineral gain results agreed with the microhardness findings, which also showed that the 0.1 μM solution had the highest increase in enamel hardness after treatment. In combination, these findings suggest that 0.1 μM AKBA showed maximum effectiveness for remineralization of both the mineral level and mechanical strength of enamel when compared to other concentrations evaluated.

Results

Enamel microhardness

According to the results of the ANOVA test, there were no significant differences in surface microhardness values among all groups at the baseline and demineralization phases ($p > 0.05$), while there were significant statistical differences among the four groups in the remineralization phase, as revealed in Table 2.

Table 2. Descriptive and statistical test repeated measure one-way ANOVA of surface microhardness in (kg/mm^2) at the three time periods for every remineralizing agent.

		Baseline	Demin	Treat	F	p value
0.01	N	15	15	15	811.368	0.000
	Minimum	223.400	143.500	268.900		
	Maximum	331.600	148.600	304.900		
	Mean	288.800	146.045	291.289		
	$\pm\text{SD}$	54.157	2.133	12.706		
0.1	N	15	15	15	931.541	0.000
	Minimum	322.600	143.170	290.830		
	Maximum	328.400	147.700	318.030		
	Mean	326.413	146.096	300.227		
	$\pm\text{SD}$	1.910	1.655	9.768		

1	N	15	15	15	891.091	0.000
	Minimum	215.900	143.870	290.800		
	Maximum	331.500	149.170	311.530		
	Mean	301.620	146.640	298.581		
	±SD	51.845	2.137	8.935		
F		1.560	0.220	1.613		
p value		0.234	0.804	0.223		

Table 2 reports the mean enamel surface microhardness values at baseline (sound surface), demineralization, and after treatment. Following demineralization, all groups showed a significant decline in enamel microhardness relative to baseline after chemical remineralization therapy.

With the Bonferroni Honestly Significant Difference (Bonferroni) test, Table 3 displays numerous pairwise comparisons of microhardness throughout phases. The demineralization phase and the remineralization phase differ significantly ($p < 0.05$). In contrast to the remineralization phase, all concentration mean differences during the baseline phase were larger ($p < 0.05$).

The 0.1 μM AKBA had the highest microhardness value, while other concentrations had a lower value than it (Figure 2).

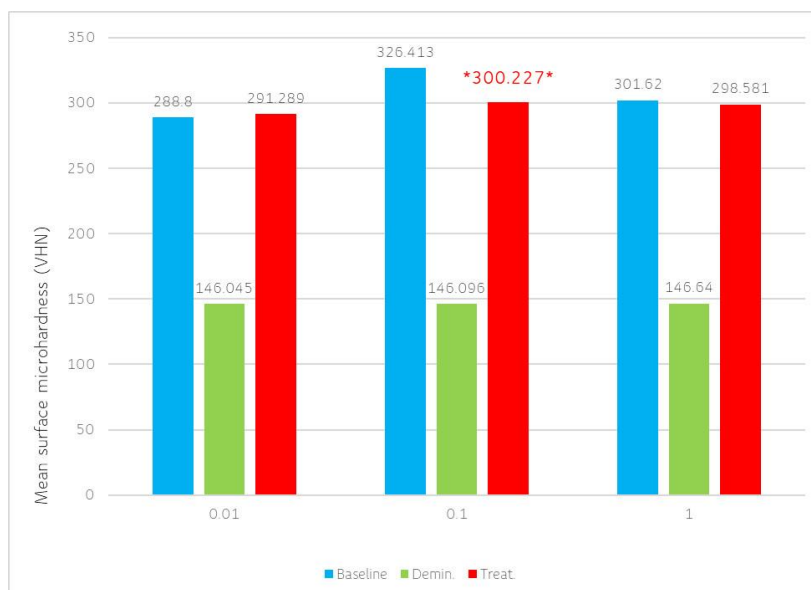


Figure 2. Bar graph of surface microhardness for each concentration at different phases.

Table 3. Multiple pairwise comparison of surface microhardness among phases using Bonferroni.

Conc.	Phases		Mean difference (I-J)	p value
0.01	Baseline	Demin.	142.755	0.000
		Treat.	-2.489	1.000
	Demin.	Treat.	-145.244	0.000

0.1	Baseline	Demin.	180.316	0.000
		Treat.	26.185	0.306
	Demin.	Treat.	-154.131	0.000
1	Baseline	Demin.	154.980	0.000
		Treat.	3.039	1.000
	Demin.	Treat.	-151.941	0.000

The 0.1 μ M AKBA had the highest microhardness value, while the other concentrations had a lower value than it.

Microscopic feature of the enamel surface by using Filed Emission Scanning Electron Microscopy (FESEM)

The enamel surfaces treated with different concentrations of AKBA showed noticeable variations in surface morphology and mechanical behavior, as displayed by FESEM analysis and corresponding microhardness measurements.

Energy-Dispersive X-ray spectrometry (EDX)

The data obtained by EDX analysis for every group regarding the weight percentage of calcium (Ca) phosphate (PO₄). A lower mean weight percentage for Ca and PO₄ was observed during demineralization. Both elements' weight percentages increased following remineralization with the AKBA group.

Descriptive and statistical tests of Ca and PO₄ in the AKBA group

See Table 4.

Table 4. Descriptive statistics of % atomic of calcium and phosphorus among groups.

Groups		Baseline P	Baseline Ca	Dem. P	Dem. ca	Treat. P	Trea. ca
0.01	Minimum	11.200	21.300	5.500	8.900	9.800	19.800
	Maximum	11.400	21.600	7.800	14.500	10.000	21.000
	Mean	11.300	21.433	6.667	12.233	9.900	20.467
	$\pm$ SD	.100	.153	1.150	2.948	0.100	.611
0.1	Minimum	11.300	21.200	5.200	8.900	10.000	19.900
	Maximum	11.400	21.800	7.200	14.400	11.100	21.800
	Mean	11.367	21.433	6.500	12.233	10.533	20.767
	$\pm$ SD	.058	.321	1.127	2.930	0.551	.961
1	Minimum	11.200	21.200	5.400	8.800	9.500	19.600
	Maximum	11.500	21.900	7.400	14.500	10.200	20.800
	Mean	11.367	21.500	6.667	12.233	9.867	20.333
	$\pm$ SD	.153	.361	1.102	3.024	.351	.643

F and p value for treatment phosphorous is 2.908 and 0.131, while F and p value for treatment phosphorous is 0.259 and 0.780.

Descriptive analysis showed a reduction in calcium and phosphorus atomic percentages after the demineralization phase across all groups. Following the treatment phase, an improvement in both elements was seen, with noticeable variation among the tested concentrations. The 0.1 μ M group displayed the most favorable remineralization response, revealing a greater increase in calcium levels and better recovery in phosphorus compared to the 1 μ M and 0.01 μ M groups.

Discussion

Earlier studies have exhibited that bioactive materials can improve enamel microhardness and remineralization (Al-Ward & Radhi, 2023; Alateya et al., 2025). Similarly, the present study proposes that AKBA may act as a bioactive agent promoting enamel reinforcement and remineralization.

Herbal-based extracts have proved a protective effect on enamel by lowering mineral loss and enhancing resistance to acidic challenges. In prior studies, siwak water extract, particularly at higher concentrations, significantly reduced calcium ion dissolution compared to deionized water, advocating improved enamel resistance (Hoobi et al., 2014).

This study aimed to observe the effect of varying concentrations of Acetyl-11-keto- β -Boswellic acid (AKBA) on enamel microhardness and surface topography in an initial caries lesion. After demineralization, all groups exhibited a significant weakening in enamel microhardness values, showing effective production of initial caries lesions (Figure 1 b). Following the remineralization phase, the microhardness of enamel treated with 0.1 μ M AKBA exceeded that of the 1 and 0.01 μ M groups, demonstrating greater improvement in surface hardness.

The FESEM analysis showed clear differences in enamel surface morphology among the tested concentrations. The 0.1 μ M AKBA-treated enamel displayed a smooth, continuous, and well-organized surface with minimal particle aggregation (Figure 1d). In contrast, the 1 μ M group showed a rough and uneven surface with high particle aggregation (Figure 1c), whereas the 0.01 μ M group showed insufficient and discontinuous surface coverage with scattered particles (Figure 1e).

Analysis by EDX showed that 0.1 μ M had the highest remineralization where, compared to the demineralized state, more calcium and phosphate were restored. This proves that AKBA at this concentration had the ability to facilitate in the remineralization process and support the structure of the enamel (Table 4).

Previous studies have reported antibacterial activity of AKBA against oral pathogens, including *Streptococcus mutans* and *Lactobacilli* (Raja et al., 2011). This reported activity may represent a possible biological mechanism associated with the effects of AKBA; however, antibacterial activity was not directly evaluated in the present study and therefore cannot be considered a direct explanation for the observed remineralization.

The present findings are also supported by Aldandan et al. (2024), who reported that AKBA enhanced the mineralization potential of bone marrow mesenchymal stem cells by upregulating osteogenic markers and promoting mineral deposition. Although enamel and bone are different tissues, both contain hydroxyapatite as a major mineral component. Therefore, the mineralization-promoting activity reported for AKBA may provide supportive evidence for the mineral recovery observed in the present study.

Limitation

The study was achieved in an in vitro environment using artificial saliva and controlled conditions, which may not completely replicate the complexities of the oral environment. Factors such as salivary composition, pH variations, and the presence of bacteria, which enhance enamel remineralization in vivo, are not reviewed.

Recommendations

Further in vivo and clinical studies are suggested to estimate the effectiveness of AKBA under oral conditions. Long-term studies should also be conducted to evaluate the durability and stability of the remineralized enamel. In addition, the development of clinical delivery systems, such as gels, varnishes, or toothpastes having AKBA, is suggested. Further examinations using advanced analytical techniques, including FTIR, Transmission Electron Microscopy (TEM) and Raman spectroscopy, are recommended to assess enamel surface and structural changes. Micro-computed tomography may also be used to assess the penetration depth of AKBA into enamel lesions. Finally, future studies should investigate the effect of AKBA on dentin remineralization in addition to enamel.

Conclusion

Based on the in vitro data of this study, AKBA seems to be a promising remineralizing agent for the management of initial enamel caries. The effectiveness was concentration-dependent, with 0.1 μM concentrations providing accumulation of superiority in the increase of enamel microhardness, the preferred morphology, and the better recovery of calcium and phosphate mineral content. This concentration, which affected the formation of a homogeneous and well-adhered surface layer, was found to be significant in the reinforcing effect and enamel-repair ability. Accordingly, 0.1 μM AKBA may be the optimal concentration for enamel remineralization among all the concentrations examined here.

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Declaration of Competing Interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of Conflicting Interests

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