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(RESEARCH ARTICLE)

Effectiveness of Etawa goat milk casein paste on increasing permanent tooth enamel hardness

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Abstract

Demineralization is the process of mineral solubility that affects the hardness of tooth enamel. Etawa goat milk casein contains bioactive peptides that can enhance the hardness of tooth enamel through remineralization, which is the restoration of dissolved minerals. This study aims to determine the effectiveness of etawa goat milk casein paste on increasing permanent tooth enamel hardness. The method employed in this research involved applying etawa goat milk casein paste with concentrations of 10%, 20%, and 30% respectively to the buccal surface of the first permanent upper premolar teeth that had been demineralized. Subsequently, the teeth were immersed in artificial saliva and placed in an incubator at 37°C for a 24-hour interval. The treatment was conducted over a period of 14 days. Pretest and post-test enamel hardness measurements were performed using a digital Microhardness Vickers Test. There was an increase in the hardness value of permanent tooth enamel following the application of etawa goat milk casein paste. One Way ANOVA and Pearson correlation tests indicated significant results. Etawa goat milk casein paste is effective in increasing permanent tooth enamel hardness.

Keywords: Etawa goat milk casein; Enamel hardness; Permanent teeth; Remineralization; Microhardness Vickers Test

1. Introduction

Dental caries is a disease caused by the production of cariogenic bacterial acids. Enamel is the hardest substance in the human body and serves as the outer layer of the tooth crown, resistant to wear. When the pH on the enamel surface decreases, hydrogen ions attack hydroxyapatite and dissolve calcium (Ca) and phosphate (P) components, leading to demineralization and degradation of enamel as well as a decrease in the force-bearing function of enamel. Efforts to prevent the occurrence and development of caries require inhibiting cariogenic bacteria and restoring dissolved minerals through remineralization [1].

The calcium, phosphate, and casein present in milk are capable of inhibiting demineralization and enhancing tooth remineralization [2]. Milk is a fluid produced by the mammary glands of mammals. The composition of milk varies according to the species. Cow's milk contains 20 allergenic proteins that can cause allergic reactions, especially in individuals with atopic diseases [3, 4]. Goat's milk contains higher levels of total protein, casein, milk fat, minerals, and vitamin A compared to cow's milk. Goat's milk is a source of easily digestible peptides, is well absorbed, and has lower allergenic properties. Additionally, goat's milk contains higher levels of total protein, casein, milk fat, minerals, and vitamin A compared to cow's milk [5].

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In a previous study conducted by Sutanti et al [6], it was demonstrated that Etawa goat milk casein gel was effective in enhancing the hardness of primary tooth enamel. Furthermore, the research results also indicated that Etawa goat milk casein possesses antioxidant activity and can act as an antibacterial agent against *Streptococcus mutans*. However, the results of this Etawa goat milk casein gel were not homogeneous. One of the contributing factors to the lack of homogeneity in the preparation is the instability of mixing, resulting in poor uniformity of all components. Other factors such as solubility, type and quantity of solvent, and interactions between solvent and materials can also influence the homogeneity of a preparation [7].

In this study, the researchers proposed a paste formulation as a topical application of Etawa goat milk casein on permanent tooth enamel. The primary reason for using paste formulation is due to its stronger adhesive properties compared to other formulations such as gels and ointments. Paste is more readily absorbed and allows for longer contact with the application site, thereby maximizing substance release. Additionally, paste can create a protective barrier, thus safeguarding the mucosa from irritation [8].

2. Material and methods

A total of 8 liters of Etawa goat milk were subjected to casein isolation, resulting in the formation of precipitates which were then dried into powder form. The produced casein powder was subsequently utilized to prepare paste formulations with concentrations of 10%, 20%, and 30%.

The research sample consisted of 25 first permanent upper premolar teeth that were extracted based on inclusion criteria, including absence of caries, crown fractures, enamel and dentin damage, and hypoplasia. Exclusion criteria for the samples involved teeth experiencing damage during the study or fractures resulting from the administered treatments.

The extracted samples were preserved in 2% formaldehyde (pH 7.0), then cleaned with a brush and pumice, and subsequently immersed in normal saline solution to maintain sample quality. Permanent teeth with remaining roots were cut mesiodistally at the cemento-enamel junction (CEJ) using a low-speed micromotor with a separating disk bur, leaving only the crown portion. The crown surfaces were sterilized with ethylene oxide.

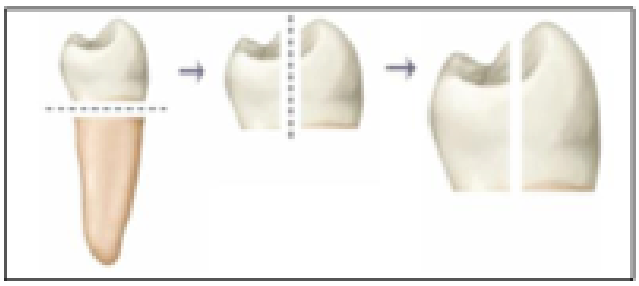

Figure 1 The cutting of the tooth crown [9]

All crown surfaces of the teeth (except for the flat and widest buccal surfaces) were mounted by embedding them in self-curing acrylic with the buccal aspect facing upwards [9]. The samples were polished using a rotary grinder and polisher, followed by demineralization using a 37% phosphoric acid solution for 20 minutes. Subsequently, the samples were rinsed with water and dried [10]. The enamel hardness of all demineralized permanent tooth samples was then measured (pre-test) using a Digital Microhardness Vickers Test.

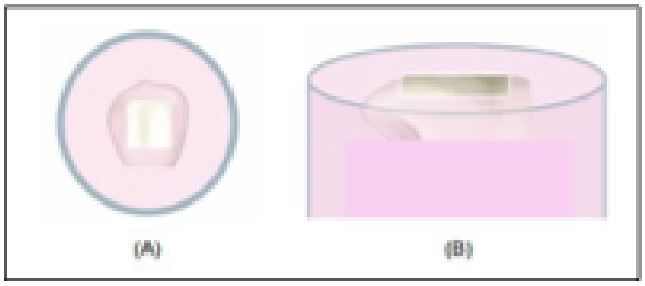

Figure 2 Embedding of tooth samples in self-curing acrylic (A) from the top (B) from the side [9]

The pre-tested samples were then divided into treatment groups, with each group comprising 5 samples: KP (CPP-ACP (GC-Tooth Mousse®)), KN (distilled water), KP1 (Etawa goat milk casein paste 10%), KP2 (Etawa goat milk casein paste 20%), and KP3 (Etawa goat milk casein paste 30%).

The teeth samples in the treatment groups were evenly coated with the respective Etawa goat milk casein paste for 5 minutes, then rinsed with distilled water and dried. This treatment was administered once daily with a 24-hour interval, during which the samples were immersed in artificial saliva and incubated at 37°C. The testing of the test material on the tooth samples was conducted continuously for 14 consecutive days [10, 11]. Subsequently, all samples were subjected to enamel hardness testing again (post-test) using a Digital Microhardness Vickers Test.

3. Results and discussion

An increase in enamel hardness values occurred in all treatment groups. The Etawa goat milk casein paste group with a concentration of 30% yielded the highest difference in increased permanent tooth enamel hardness values, followed by the Etawa goat milk casein paste group with a concentration of 20%, concentration of 10%, then the positive control group CPP-ACP (GC-Tooth Mousse®), and finally the negative control group (distilled water).

Table 1 Enamel hardness of permanent teeth

Group	**Enamel hardness of permanent teeth (*Mean*)**		***Delta* (Δ)**
	Pretest (VHN)	*Post-test* (VHN)	
CPP–ACP (GC-Tooth Mousse®) (KP)	368.900	393.240	24.340
Distilled water (KN)	363.860	373.000	9.140
Casein 10%	370.980	396.340	25.360
Casein 20%	362.380	402.520	40.140
Casein 30%	366.280	412.720	46.440

In this study, the mean variation of enamel hardness values after demineralization (pretest) ranged from 362 to 370 VHN. This variation in enamel hardness values was higher than the initial enamel hardness values reported by Salazar et al [12] in their study, which ranged from 250 to 360 VHN. This difference could be influenced by several factors, including variations in the histological aspects of teeth and the chemical structure of enamel in each permanent tooth sample. Additionally, the demineralization agent used, 37% phosphoric acid, also played a role. The type of 37% phosphoric acid gel used in this study resulted in partial demineralization of the enamel surface, but due to its high viscosity, it could not penetrate deeply. This influenced the decrease in enamel hardness produced [13].

According to Abbasoglu et al [14], the increase in enamel hardness values is caused by the mechanism of the remineralization process, which is influenced by the effectiveness of the remineralization agent on each tooth sample. The effectiveness of the remineralization agent is also influenced by several factors, including differences in concentration, solubility of the material, acidity level (pH), temperature, formation of complex reactions, equilibrium point positions, and chemical formulation of the remineralization material [15].

The comparison of enamel hardness values after demineralization (pretest) and after treatment with Etawa goat milk casein paste (post-test) showed a difference. This indicates an increase in all treatment groups with Etawa goat milk casein paste, as well as in the positive control group CPP-ACP (GC-Tooth Mousse®) and the negative control group distilled water. Thus, the researchers concluded that Etawa goat milk casein paste can increase enamel hardness values after demineralization. This increase in enamel hardness values was statistically significant, as demonstrated by the One-Way ANOVA test with a significance level of 0.001 (<0.05).

Tabel 2 Result of paired t-test

Group	Enamel Hardness		Sig.
	Pre	Post	
CPP–ACP (KP)	368.900 ± 38.976	393.240 ± 4.938	0.196
Distilled water (KN)	363.860 ± 18.856	373.000 ± 7.403	0.432
Casein 10%	370.980 ± 20.008	396.340 ± 14.669	0.004*
Casein 20%	362.380 ± 24.031	402.520 ± 5.607	0.035*
Casein 30%	366.280 ± 20.693	412.720 ± 20.507	0.005*

*p < 0.05

Based on paired t-test, an increase in enamel hardness was observed, but not statistically significant in the CPP-ACP and distilled water control groups. Conversely, the treatment groups with 10%, 20%, and 30% casein showed a significant increase in enamel hardness. Both CPP-ACP (GC-Tooth Mousse®) and Etawa goat milk casein are based on calcium and phosphate ions. Both substances function to inhibit enamel lesions caused by demineralization by enhancing remineralization [16].

The demineralization and remineralization processes constitute a cycle that targets the hard tissues of the teeth, particularly enamel, and focus on calcium and phosphate. To enhance enamel remineralization under cariogenic conditions, bioactive agents derived from dairy products have been developed. Milk and dairy products, as natural products, are recommended due to their nutritional content, particularly calcium and phosphate, serving as protective agents [17].

Goat milk is rich in protein, vitamins, fats, and minerals, often dubbed as "The king of milk". The protein content in goat milk is exceptionally high, reaching up to 85%. Goat milk protein consists of whey and casein. Casein in goat milk is considered safer for consumption and free from anaphylaxis [18, 19].

Casein Phospho-peptide (CPP) is a peptide derived from the β-casein fraction of goat milk casein. β-casein, comprising 30% of total milk protein and 48% of total casein, constitutes the largest fraction compared to other casein fractions [20]. CPP interacts with calcium, phosphate, and fluoride ions to produce an amorphous phase (Amorphous Calcium Phosphate/ACP) through enzymatic processes, forming CPP-ACP. Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) is saturated and provides Ca^{2}+ and $PO4^{2}$- ions for enamel remineralization [21].

CPP-ACP has the ability to stabilize calcium and phosphate ions on tooth enamel. The application of CPP-ACP reduces surface softening and morphological changes in porous teeth by forming a layer that fills the interprism spaces and partially covers the prisms to protect them from acid dissolution [22]. Oshiro et al.'s study [23] concluded that CPP-ACP only partially alters the morphological structure of enamel. However, CPP-ACP still prevents demineralization of tooth structure. Meanwhile, Poggio et al. [24] stated that CPP-ACP is effective in preventing dentin/enamel erosion. However, clinically and statistically significant protective effects were only observed on the enamel surface. This is because CPP-ACP forms a layer that fills the enamel inter-prism cavities and partially covers the prisms for a long period, thus preventing acid contamination that leads to enamel lesions.

This study utilized artificial saliva for soaking tooth samples. During the remineralization process, artificial saliva is needed to mimic the oral cavity environment because remineralization agents must come into contact with saliva to enhance remineralization [21]. Meanwhile, distilled water is pure mineral-free water obtained through mineral water distillation, meaning it has no remineralization effect on tooth samples as it lacks any mineral content. However, the soaking process in artificial saliva containing minerals similar to those found in normal saliva results in micro-hardness enhancement in enamel [15].

The Post Hoc Tukey test revealed significant differences between the negative control group distilled water and all treatment groups with Etawa goat milk casein paste. The increase in enamel hardness values in all casein concentration groups was significantly higher compared to the distilled water group. Meanwhile, with the positive control CPP-ACP, there was no significant difference compared to all other groups. Thus, it can be concluded that the positive control CPP-ACP also provides an effect on increasing permanent tooth enamel hardness, although not as much as the effect provided by Etawa goat milk casein paste.

Tabel 3 Post Hoc Tukey test

		P Value
CPP–ACP (KP)	Distilled water (KN)	0.104
	Casein 10%	0.994
	Casein 20%	0.751
	Casein 30%	0.125
Distilled water (KN)	Casein 10%	0.047*
	Casein 20%	0.008*
	Casein 30%	0.000**
Casein10%	Casein 20%	0.928
	Casein 30%	0.251
Casein 20%	Casein 30%	0.682

** p < 0.001; * p < 0.05

The increase in permanent tooth enamel hardness values by Etawa goat milk casein paste was also analyzed for correlation using Pearson. The test results yielded a significance value of 0.000, indicating a significant correlation between the application of Etawa goat milk casein paste with concentrations of 10%, 20%, and 30% and the delta increase in permanent tooth enamel hardness values. The correlation value obtained was positive (r=0.665), meaning that the higher the concentration of Etawa goat milk casein paste, the higher the permanent tooth enamel hardness values.

4. Conclusion

Based on the study results, it can be concluded that Etawa goat milk casein paste is effective in increasing the hardness of permanent tooth enamel. The higher the concentration of Etawa goat milk casein paste, the greater the increase in the hardness of the permanent tooth enamel surface.

Compliance with ethical standards

Disclosure of conflict of interest

The authors report no conflicts of interest to declare

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