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Utilizing Hydroxyapatite Remineralization Paste Derived from Snakehead Fish Bone Meal (*Channa striata*) Enhances the Hardness of Dental Enamel

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Abstract

Dental caries can be prevented through tooth remineralization. To achieve this, the innovation of cost-effective remineralization paste using readily available waste materials is crucial. This study aims to investigate the impact of hydroxyapatite-based remineralization paste derived from snakehead fish bones (channa striata) on dental enamel hardness, thereby contributing to tooth remineralization efforts. This research adopts an experimental approach, involving 48 extracted teeth divided into four groups of 12 teeth each. The teeth were treated with hydroxyapatite remineralization paste sourced from snakehead fish bones for a duration of 3 minutes, twice daily over a span of seven days, followed by immersion in artificial saliva. Subsequently, a Vicker's Hardness Tester was employed to assess enamel hardness. Statistical analysis was conducted using One Way ANOVA. The group treated with 50% hydroxyapatite (HA) paste demonstrated the highest average hardness. The maximum standard deviation was observed in the 30% group, recording a value of 65.72. The outcomes of the one-way ANOVA test underscored a significant effect of HA remineralization paste application on dental enamel hardness (p = 0.000). Post-hoc analysis using LSD revealed no significant difference in p-values (> 0.05) between the 10% and 30% groups. The most substantial mean difference was identified between the control group and the 50% group. The utilization of remineralization paste effectively enhances dental enamel hardness, advocating for the enhancement of dental health professionals' skills to facilitate the provision of such paste to patients.

Keywords: Remineralization, hydroxyapatite, snakehead fish bone, dental enamel

Full-length article
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1. Introduction

Demineralization is the process involving the loss of minerals at the forefront of the lesion beneath the enamel surface. It entails the transportation of acid ions from the plaque to the lesion and the movement of mineral ions from the front of the lesion to the plaque [1]. The remineralization process is a natural repair mechanism that restores minerals, in ionic form, to the hydroxyapatite (HAP) crystal lattice [2]. This process occurs under physiological pH conditions close to neutral, wherein calcium and phosphate mineral ions are redeposited into the caries lesion from saliva and plaque fluid. This results in the formation of newer, larger, and more resistant HAP crystals [3]. Demineralization and remineralization are continuous dynamic processes that occur to some extent on hard tissue surfaces. As a result, they significantly impact the health of these hard tissues, with the surrounding environment playing a crucial role. Therefore, the main goal is to maintain an environment that prevents demineralization while promoting remineralization [4]. Maintaining good oral health is significantly achievable through prevention and early treatment. The predominant concern is dental caries, which constitutes the majority of cases. On a global scale, approximately 2 billion individuals experience caries on their permanent teeth, while 514 million children are affected by caries on their primary teeth [5]. The incidence of dental caries is increasing, attributed to urbanization and changing living conditions, such as a shift in dietary habits [6]. This escalation can be attributed to insufficient exposure to fluoride, stemming from factors like inadequate water supply fluoridation and lack of fluoride-rich oral hygiene products like toothpaste. Additionally, the ready availability of sugary foods, along with the consumption of tobacco and alcohol, further contribute to this trend [7]. Hence, it is imperative to undertake proactive measures to avert the onset of caries in children. Preventive actions that prove effective in addressing early-stage caries encompass the application of topical fluoride to the tooth's surface [8].

Teeth consist of HA, denoted as $Ca_{10}(PO_4)_6(OH)_2$, which are rich in calcium and phosphate. HA proves highly effective as a remineralization agent for tooth enamel. This efficacy arises from its capacity to reconstitute apatite crystals [9]. HA stands as a primary constituent within bones and teeth. Enamel, for instance, comprises HA, water, and various organic components. Similarly, dentin is made up of HA crystals, collagen fibers, proteins, and water [10]. HA possesses significant chemical properties that render it biocompatible, bioactive, and bioresorbable. the body and contributes to the formation of new bone tissue within the existing bone structure [11]. HA demonstrates exceptional biocompatibility with hard tissue and exhibits bioactivity in both reconstructing damaged bone tissue and interacting with soft tissue [12]. HA is often implanted as a bone substitute or filler [13] due to its similarity to the chemical structure of human bones and hard tissue. The HA found in human bones typically ranges in size from 20 to 80 nm [14]. HA can be generated from both inorganic and organic sources. For instance, inorganic sources include fish bones and beef bones [15].

Fish bones are a type of waste generated by the fish processing industry. They possess the highest calcium content among various fish body parts, primarily due to their composition of calcium, phosphorus, and carbonate, which are the main elements comprising fish bones [16]. The primary minerals found in fish bones are calcium and phosphorus. Additionally, smaller amounts of other minerals such as sodium, magnesium, and fluoride are present [17]. The potential of fish bone waste has not been fully realized, as communities and the fishing industry tend to prioritize fish meat over other parts like fish bones. Consequently, fish bone waste holds significant promise as an ingredient for dental remineralization paste, rich in calcium and phosphate. The current high cost of remineralization paste necessitates innovative approaches to make it more affordable and effective for dental care.

2. Materials and Methods

The research employed a purely experimental design, utilizing a pre-test and post-test approach to assess the impact of HA remineralization paste from snakehead fish (channa striata) bone meal on the hardness of tooth enamel. The study was conducted at the Integrated Research Laboratory of the Faculty of Dentistry at Gadjah Mada University. This study focused on the buccal aspect of the first permanent premolar in the maxilla, which had been extracted. The inclusion criteria were caries-free teeth and absence of enamel abnormalities. A minimum of 80 samples was required for each of the four groups included in this study. The sample was divided into four groups, each containing 20 tooth samples. The dependent variable in this study is the hydroxyapatite-based tooth remineralization paste derived from snakehead fish bone meal (Channa striata) at concentrations of 10%, 30%, and 50%. The independent variable is the hardness of the tooth enamel, assessed through hardness testing using the Vickers Microhardness Tester.

2.1. Research Tools and Materials

Tools: Micromotor, handpiece, Carborundum disk, diamond bur, cylindrical mold made from a syringe, acrylic cup, sandpaper with roughness sizes 400, 1000, and 1500, incubator, container for sample soaking, microbrush, Vickers Microhardness Tester. Materials: Eighty extracted maxillary first premolars, distilled water (aquades), artificial saliva, self-cured acrylic resin, separating agent (CMS).

Biocompatibility entails its ability to seamlessly integrate with the recipient's body, while bioactivity allows it to seamlessly merge with human bones. In the case of bioresorbability, the material is absorbed by

2.2. Methods of Procedure

Methods of procedure this research is: 1) Subject Selection: A total of 80 maxillary first premolars were meticulously chosen based on predetermined criteria; 2) Dental Crown Division: The dental crown was separated from the tooth root by making a horizontal cut at the cementoenamel junction (CEJ) (refer to Figure 1). The carborundum disk was utilized to perform the cutting of the premolar roots. Dental Crown Implantation: The sample container was fashioned using self-cured acrylic resin. The acrylic resin was thoroughly mixed and subsequently placed into a cylindrical mold measuring 2 cm in diameter. While the acrylic resin was still malleable, the palatal section of the sample was pressed into the resin, ensuring that the buccal opening was oriented towards the surface. Allow ample time for the acrylic resin to solidify.

2.3. Grouping of Research Participants

The research sample was divided into: 1) Group I comprised individuals subjected to the application of a 10% hydroxyapatite remineralization paste derived from snakehead fish bone meal (Channa Striata); 2) Meanwhile, Group II consisted of individuals treated with a 30% hydroxyapatite remineralization paste sourced from snakehead fish bone meal (Channa Striata); 3) Group III encompassed individuals treated with remineralization paste containing 50% hydroxyapatite derived from snakehead fish bone meal (Channa Striata). Finally, 4) Group IV was the control group, comprising 20 samples, with each sample container being assigned a unique serial number on its surface. Measuring Permanent Tooth Enamel Hardness: The hardness of the enamel was assessed using the Vickers Hardness Test. The procedure involved placing the acrylic cylinder onto the tool table, ensuring that the enamel surface was facing upwards. The magnification was set at 40x, and the test duration was 10 seconds, while the load applied was 1.96N (200 grams). The sample was positioned directly beneath the penetration diamond, which would create an indentation on the enamel surface for 10 seconds. The resulting hardness value would then be displayed on the tool.

2.4. Provision of Remineralization Materials

The sample was divided into five groups: 1) Group I: HA remineralization paste containing snakehead fish bone meal (Channa striata) at a concentration of 10% was applied. The remineralization material was loaded into a syringe (P syringe), and 0.02 ml was carefully extracted for each sample. The material was then evenly spread across all enamel surfaces using a micro brush, and left undisturbed for 3 minutes. Subsequently, the samples were immersed in artificial saliva (100 ml per group) at a temperature of 37°C. This procedure was repeated twice daily for a duration of seven days; 2) Group II: A 30% HA paste derived from snakehead fish bone meal (Channa striata) was used for this group. The remineralization material was placed into a microliter syringe, and 0.02 ml was extracted for each sample. After application with a micro brush, the material was allowed to rest on the enamel surfaces for 3 minutes. The samples were then subjected to immersion in artificial saliva (100 ml per group) at a temperature of 37°C. This process was carried out twice daily over a span of seven days; 3) Group III: In this group, a 50% HA remineralization paste from snakehead fish bone meal (Channa striata) was employed. The material was drawn into a microliter syringe, with 0.02 ml extracted for each sample. Application was performed using a micro brush to cover all enamel surfaces. After a 3-minute waiting period, the samples were placed into artificial saliva (100 ml per group) at a temperature of 37°C. Similar to the other groups, this procedure was conducted twice daily for a week; 4) Group O (Control Group): This control group was treated differently. The samples were coated with a paste devoid of HA, followed by immersion in artificial saliva (100 ml per group) for seven days. This incubation took place at a constant temperature of 37°C; 5) Evaluating enamel hardness following application of HA remineralization Paste from snakehead fish bones (channa striata): he hardness of the enamel was assessed using the Vickers Hardness test.

2.5. Data Analysis

The data collected in this study consists of numerical values representing enamel hardness. The data analysis was conducted using parametric tests, preceded by evaluations for data normality through the Shapiro-Wilk test ($p > 0.05$), as well as assessments for data homogeneity using Levene's Test of Variance ($p > 0.05$). The subsequent analysis employed the One-way ANOVA test ($p < 0.05$). his part should contain sufficient detail to reproduce reported data. It can be divided into subsections if several methods are described. Methods already published should be indicated by a reference, only relevant modifications should be described. This section should be written concisely in detail by maintaining continuity of the texts.

2.6. Ethical Approvals

This research has undergone an ethical review conducted by the Palembang Ministry of Health Polytechnic, with reference number 470/KEPK/Adm2/X/2021.

3. Results and Discussions

Referring to Table 1, it becomes evident that the group utilizing 50% hydroxyapatite paste showcases the highest average. Additionally, the group employing 30% hydroxyapatite paste demonstrates the highest standard deviation, amounting to 65.72. The impact of applying hydroxyapatite remineralization paste on tooth enamel hardness is further illustrated in table 2. Based on the outcomes of the one-way ANOVA test, the obtained p-value is 0.000, indicating a significant difference in enamel hardness between the various groups. This suggests that the administration of hydroxyapatite remineralization paste indeed influences tooth enamel hardness. Further details from the LSD Post-hoc analysis is presented in table 3. Derived from the findings presented in table 3, it becomes apparent that no distinction exists between the 10% and 30% groups, as indicated by a p-value greater than 0.05. The most notable mean difference is observed between the control group and the 50% group. HA synthesized from mackerel fish bones can be formulated into toothpaste, and the optimal formulation was found to be F1, containing a 50% concentration of HA [18]. Snakehead fish bones inherently contain calcium, thus harboring the potential to serve as a source of Calcium Oxide (CaO). The calcination process of fish bones is conducted at a temperature of 900°C. The resultant CaO nanoparticles meet the criteria for nanomaterials and hold promise as a foundational material for hydroxyapatite production [19]. Some studies require heating using infrared radiation [20]. Fish bone powder is subjected to high-temperature calcination, leading to a Ca/P molar ratio that closely approaches the stoichiometry of pure HA. However, the calcined Ca/P molar ratio exceeds the 1.67 HA stoichiometry due to the influence of carbonate and the presence of trace ions that can stimulate bone growth [21]. Research results demonstrate that infrared heating has the ability to attract water and promote the growth of new cells [22]. A Ca/P molar ratio of 1.71 demonstrate that the resulting nano-hydroxyapatite possesses significant antibacterial properties against both *Escherichia coli* and *Staphylococcus aureus* [23]. The findings of this study revealed a noteworthy enhancement in the hardness of permanent tooth enamel subsequent to the application of HA paste. Over the past decade, Hydroxyapatite nanoparticles (HAnps) have been extensively studied in various dental applications, including oral implantology, bone reconstruction, restorative dentistry, and preventive dentistry. These nanoparticles exhibit a remarkable ability to remineralize early enamel lesions and are frequently incorporated as additives to enhance existing dental materials. They find extensive utilization, particularly in preventive dentistry, as well as in restorative and regenerative practices. The literature indicates that HAnps possess exceptional physical, chemical, mechanical, and biological properties, rendering them highly suitable for a diverse array of interventions across various dental science disciplines [10]. Tests were conducted on five groups of fishbone ratios : 0% (control group), 5%, 10%, 15%, and 20%. These tests ran for periods of 3, 6, 24, and 72 hours. The results indicated that higher doses of fish bones and longer contact times were more effective in absorbing Pb, resulting in the highest Pb removal efficiency. Specifically, after a 72-hour washing process with a 20% dose of fish bones, the condition reached a Pb removal efficiency of 24.76% [24]. According to the ANOVA test, it is evident that the group receiving a 50% concentration of hydroxyapatite paste exhibited the highest level of hardness in permanent tooth enamel compared to the control group. In terms of physico-functional and mechanical properties related to biocompatibility and osteogenesis, collagen-hydroxyapatite composites induce higher ALP (alkaline phosphatase) levels. However, the incorporation of chitosan and HA increases stiffness and degradation rates while decreasing water-holding capacity and scaffold porosity [15]. Hydroxyapatite derived from snakehead fish (Channa striata) bone meal is rich in phosphate and calcium ions, facilitating remineralization. This is supported by other research indicating that providing biscuits modified with Moringa leaves and snakehead fish has led to a significant improvement in nutritional status [25]. The remineralization process of tooth enamel occurs because of the calcium and phosphate ions present, which can diffuse from the application of hydroxyapatite paste into the microporosity of the enamel. This leads to an enhancement in the hardness of the tooth enamel. Any substances applied to the enamel surface of teeth with early caries, in addition to fluoride, contribute to the process of remineralization [26].

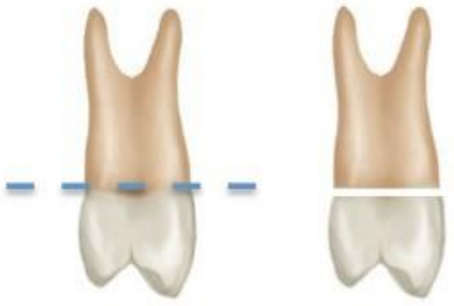

Figure 1: Dental Crown Sectioning

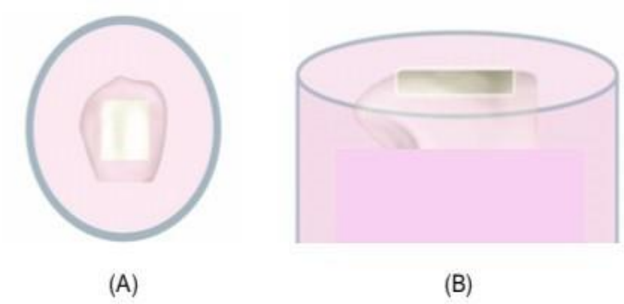

Figure 2: Dental crowns embedded in self-cured acrylic resin and leveled top view (B) side view

Table 1: Mean Enamel Hardness After Application of Hydroxyapatite Remineralization Paste Derived from Snakehead Fish Bone Meal (Channa Striata)

No.	**Email Violence**	**Mean**	**Standard Deviation**
1.	Control Group (F0)	175,86	54,69
2.	10% hydroxyapatite paste group (F1)	231,72	61,29
3.	30% hydroxyapatite paste group (F2)	263,62	65,72
4.	50% hydroxyapatite paste group (F3)	315,78	37.85

Table 2: Impact of Hydroxyapatite Remineralization Paste Application on Tooth Enamel Hardness

		n	**Mean ± SD**	**P***
Email Violence	Control Group (F0)	20	175,86 ± 54,69	0,000
	10% hydroxyapatite paste group (F1)	20	231,72 ± 61,29	
	30% hydroxyapatite paste group (F2)	20	263,62 ± 65,72	
	50% hydroxyapatite paste group (F3)	20	315,78 ± 37,85	

Table 3: LSD Post-Hoc Analysis Results

	Mean (VN)	**IK 95%**		**P***
		Minimum	**Maximum**	
control vs 10%	-55,8550	-91,065	-20,645	0,002
control vs 30%	-87,7600	-122,970	-52,550	0,000
control vs 50%	-139,9150	-175,125	-104,705	0,000
10% vs 30%	-31,905	-67,115	3,305	0,075
10% vs 50%	-84,0600	-119,270	-48,850	0,000
30% vs 50%	-52,1550	-87,365	-16,945	0,004

The process of remineralization for dental caries is a natural mechanism aimed at restoring minerals in an ionic form to the HA crystal lattice. Under nearly neutral physiological pH conditions, calcium and phosphate mineral ions are redeposited into the saliva within carious lesions. The plaque fluid that generates HA crystals is characterized by being newer, larger, and more resistant to acid dissolution (2). Innovative technology, which is based on a biomimetic regeneration system that includes HA, promotes enamel remineralization [27].

4. Conclusions

The hydroxyapatite remineralization paste derived from snakehead fish bones enhances the hardness of tooth enamel. This paste aids in the remineralization process of enamel, helping prevent dental caries. Consequently, there is a need for continuous and intensive education on the use of toothpaste derived from snakehead fish bones for maintaining dental health.

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Declaration of Interest Statement

In this article, there is no conflict of interest with any other groups.

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