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Original Article

DIFFERENCES IN THE EFFECT OF APPLYING 15% HYDROXYAPATITE PASTE FROM GOLDFISH SCALES (*CYPRINUS CARPIO*) WITH CASEIN PHOSPHOPEPTIDE–AMORPHOUS CALCIUM PHOSPHATE (CPP-ACP) AS A REMINERALIZING MATERIAL ON ENAMEL SURFACE HARDNESS

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ABSTRACT

Objective: Caries is a multifactorial disease that affects most of the world's population and is a detrimental factor for oral health. Demineralization on teeth can reduce the hardness of the enamel surface, which causes enamel to be susceptible to caries. The purpose of this study was to determine the effect of giving 15% hydroxyapatite paste from goldfish scales (*Cyprinus carpio*) with CPP-ACP as a remineralizing agent on the enamel surface hardness.

Methods: This laboratory experimental study using 24 samples of post-extraction maxillary first premolars. There were 4 groups of samples, group I samples that were not given the test material were only soaked in artificial saliva, group II samples were only demineralized with HCl solution for 120 seconds, both test groups were then demineralized with HCl solution for 120 seconds before being given the test material. Group III samples were applied with the test material CPP-ACP for 2 min; group IV samples were applied with 15% goldfish scale hydroxyapatite paste (*Cyprinus carpio*) for 2 min. Both test groups were given treatment for 14 consecutive days. All sample groups were tested for surface hardness using the Microvickers Hardness Tester.

Results: The results of the Mann-Whitney analysis test showed that there was a significant difference in the effect of pn<0.05 between each group studied. Based on the results of the study, there was an effect after administration of 15% hydroxyapatite paste of goldfish scales (*Cyprinus carpio*) with CPP-ACP as a remineralizing agent on enamel surface hardness.

Conclusion: Based on the results of research on the effect of administering 15% goldfish (*Cyprinus carpio*) scale hydroxyapatite paste with Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP-ACP) on enamel surface hardness, it can be concluded that there is a significant effect after administering goldfish (*Cyprinus carpio*) scale hydroxyapatite paste 15 % and CPP-ACP on enamel surface hardness.

Keywords: Goldfish scale, Remineralization, Hydroxyapatite, Micro vickers hardness tester

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INTRODUCTION

Caries is a multifactorial disease that affects most of the world's population and is a detrimental factor for oral health. Based on the 2018 Indonesia Basic Health Research (RISKESDAS) data, caries prevalence in the Indonesian population reaches 45.3% [1] and in research conducted by Ozdemir (2013) stated that caries is a worldwide health problem affecting 60-90% of the world's population [2]. In other words, six to nine people out of ten people get caries. The surface of the teeth is covered with biofilm, which is a layer of mucus composed of millions of bacterial cells, salivary polymers and food debris. In a facultative state, this biofilm can easily reach hundreds of cells on the tooth surface. The biofilm that forms, also known as plaque, provides an excellent attachment site for the colonization and growth of many bacterial species. The reaction of sucrose in food with bacteria on the enamel surface produces acids which cause loss of calcium from the crown enamel layer. Demineralization is the process of removing mineral ion content in hard tissues such as hydroxyapatite crystals. This is a reversible process and hydroxyapatite mineral deposition can be carried out again through the remineralization process [3]. Fish scales, which are often considered as organic waste, actually contain many very useful ingredients such as collagen, calcium and phosphorus [4]. Wang's research (2020) states that fish scales contain high levels of amelogenin, where amelogenin plays an important role in the preparation of organic matrices, especially in biomineralization of the enamel surface [5]. Based on this background, the researchers wanted to conduct a study to test 15% hydroxyapatite synthesized from goldfish (*Cyprinus carpio*) scales with Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP-ACP) as a remineralizing agent against surface hardness.

MATERIALS AND METHODS

This research is a laboratory experimental research with the Post-test Only Group Design research design, which has received ethical clearance from the Ethics Commission of the Universitas Sumatera Utara Faculty of Medicine with number 221/KEPK/USU/2023. The research sample consisted of 24 maxillary premolars that had been extracted, crowns intact, no caries, no fractures and no restorations.

Samples were divided into 4 groups. Group I was not given sample test material and only immersed in artificial saliva for 14 d. Group II were samples demineralized in HCl solution at pH 5 for 120 seconds and then soaked in artificial saliva for 14 d. Group III, samples were demineralized in HCl solution at pH 5 for 120 seconds and then CPP-ACP paste was applied consecutively for 14 d. Next, in Group IV, samples were demineralized in HCl solution at pH 5 for 120 seconds and then hydroxyapatite paste from goldfish scales was applied consecutively for 14 d.

The process for making hydroxyapatite paste is made from 160 grams of dried goldfish scales in a blender until smooth. Then put in 500 ml of 0.1M HCl for 2 h at room temperature rinsed until neutral pH. Then proceed with immersion in 5% NaOH solution and heated on a hot plate for 5 h in a beaker glass. After that, 50% NaOH was added again and heated to 100 °C while stirring with a magnetic stirrer to denature the remaining protein. After filtering, the powdered scales were dried in an oven at 60 °C for 8 h and then calcined at 900 °C to synthesize hydroxyapatite ceramics. Next, the hydroxyapatite powder is mixed with glycerol, CMC-Na, and distilled water to form a paste with a ratio of 15:85 and a concentration of 15%.

Treatment was given according to their respective groups and

stored in an incubator at 37 °C to simulate the state of the oral cavity. After 14 d, tooth surface hardness was measured at 3 points representing the upper 1/3, middle 1/3, and lower 1/3 of the crown of the tooth. After obtaining the data, statistical analysis of data was carried out.

RESULTS AND DISCUSSION

Hydroxyapatite powder synthesized from goldfish (*Cyprinus carpio*) scales was subjected to FTIR test to identify the functional groups of a sample. The results of the FTIR spectrum characterization analysis of the hydroxyapatite sample after calcination are shown in fig. 1 which produces several functional groups, namely hydroxyl (OH^-), carbonate (CO_3^{2-}), and phosphate (PO_4^{3-}). The wave range of functional groups (OH^-) ranges from 3300-3600. The group (CO_3^{2-}) ranges between 1400-1550. Meanwhile, the (PO_4^{3-}) group has a wavelength range of 950-1100. The results of the FTIR test showed that the sample was a hydroxyapatite group (fig. 1) [5]. The result of micro vickers hardness test are stated in table 1. From table 1 it can be concluded that the highest average surface hardness is in group I and the smallest surface hardness is in group II. The data from the results of the surface hardness test were analyzed using the kruskal-wallis test to see the effect after administration of the test material on the surface hardness between groups. The results of the statistical analysis test showed a p-value of 0.000, which means that there was a significant difference in surface hardness between groups. Furthermore, Mann-Whitney statistical analysis was carried out to see the significance of differences in violence between one group and another. The results of the statistical analysis test showed that the overall p-value between the small groups was 0.05, which means that there was a significant difference in surface hardness between the test groups.

In this study, the administration of the test material was carried out for 2 min and for 14 consecutive days. The timing of the administration of the test material in this study was based on the research of Shetty (2014) which stated that a significant increase in enamel surface hardness with an optimal application time occurred in the treatment group with an application time of 2 min [6]. Based on Ostwald Ripening's theory that the mechanism of crystallapatite formation will be formed through various forms of crystallographic orientation such as cubes, rods, and hexagonal, where these layers will bind to each other and form nanorods and nanoclusters which will be the beginning of the remineralization of the enamel surface [7]. Saliva can also act as a tooth remineralization agent and, inhibit tooth demineralization during periods of low pH and promote tooth remineralization when the pH returns to a neutral state. This research is in line with research by Ionta (2014) which proved that artificial saliva has the ability to stimulate remineralization email. The content of octacalciumphosphate (OCP) in saliva can be a precursor for hydroxyapatite so that saliva will help release calcium phosphate ions, thereby helping the formation of hydroxyapatite which can stimulate the remineralization of the enamel surface [3, 8].

Demineralization of enamel occurs through a diffusion process, namely the process of moving molecules or ions that dissolve into water and then hit the enamel or through saliva (if in oral conditions) due to differences in the concentration of acidity of a material on the surface and in the tooth enamel. Calcium is the main component in tooth structure and enamel demineralization occurs due to the release of calcium ions from tooth enamel by decomposition reactions [9]. Continuous demineralization will form small pores that enlarge and form lesions that were not there before. Research by Shen (2019) and Huq (2016) states that processed dairy products such as milk, cheese and butter have been shown to exhibit anti-cariogenic properties and support remineralization, especially calcium and phosphate content, which are raw materials for remineralization [10-12]. Precipitation of remineralizing materials on enamel depends on saliva, pH and remineralizing agent used. The phosphopeptide content in CPP-ACP can bind to calcium and phosphate on the nanoscale and precipitate on the enamel rod [13]. Calcium nanophosphate crystals are one of the active biological ingredients and are able to release calcium phosphate ions in saliva so that remineralization of the enamel lesions can occur. Calcium nanophosphate crystals in CPP-ACP can penetrate the lesion area, and form a reservoir containing calcium and phosphate ion deposits and serves to inhibit demineralization when cariogenic materials attack the oral cavity [14-16]. Another mechanism for preventing demineralization by CPP-ACP can be through the content of casein, which is able to release amino acids and act as a plaque buffer through bacterial catabolism.

Table 1: Enamel surface hardness on several test groups

Sample	Enamel Surface Hardness (VHN)			
	Group 1	Group 2	Group 3	Group 4
1	459.7	258.4	331.9	310.2
2	451.9	270.5	333.3	311.5
3	415.9	264.0	308.7	308.0
4	389.8	267.0	328.9	307.6
5	408.8	267.6	328.9	307.6
6	408.8	266.9	328.5	300.0

Group I was not given sample test material and only immersed in artificial saliva for 14 d. Group II were samples demineralized in HCl solution at pH 5 for 120 seconds and then soaked in artificial saliva for 14 d. Group III, samples were demineralized in HCl solution at pH 5 for 120 seconds and then CPP-ACP paste was applied consecutively for 14 d. Next, in Group IV, samples were demineralized in HCl solution at pH 5 for 120 seconds and then hydroxyapatite paste from goldfish scales was applied consecutively for 14 d

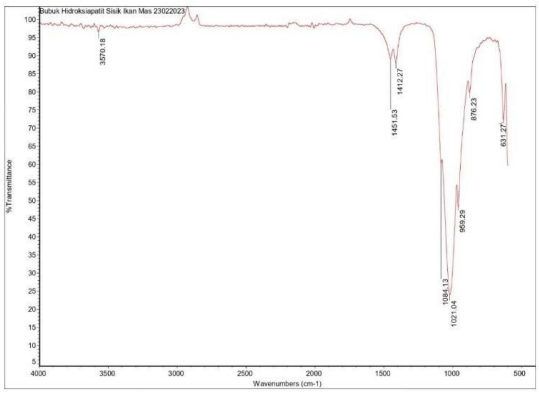

Fig. 1: FTIR of hydroxyapatite sample

From the test results of the kruskal-wallis test results obtained p value = 0.000 ($p<0.05$), which indicates that there is a significant effect in the group that is given the treatment of soaking in saliva, demineralization, application of CPP-ACP and application of hydroxyapatite paste from goldfish scales 15%.

The Mann-Whitney test was used to see the significance of the treatment in each group. Based on the Mann-Whitney test, there was a significant difference between each group that was soaked in saliva, the group that was demineralized, the group that was applied CPP-ACP and the group that was given 15% goldfish scale hydroxyapatite paste, which was evidenced by a significance value of $p = 0.000$ ($p<0.05$). The variation in surface hardness values in the average sample is thought to be related to the patient's genetics, which affects the thickness of the enamel layer, surface hardness, the age of the patient's teeth, and the duration from extraction to treatment [17]. The thicker the tooth enamel, the higher the surface hardness value. Based on the results of the above study, the value of changes in tooth enamel surface hardness produced by applying 15% goldfish (*Cyprinus carpio*) scale paste showed a significant difference; this proved that the initial hypothesis was accepted, where there was an effect of applying goldfish (*Cyprinus carpio*) scale hydroxyapatite paste 15% of the enamel surface hardness.

CONCLUSION

Based on the results of research on the effect of administering 15% goldfish (*Cyprinus carpio*) scale hydroxyapatite paste with Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP-ACP) on enamel surface hardness, it can be concluded that there is a significant effect after administering goldfish (*Cyprinus carpio*) scale hydroxyapatite paste 15 % and Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP-ACP) on enamel surface hardness.

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AUTHORS CONTRIBUTIONS

Both authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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