Hardness analysis of remineralization primary teeth enamel after the application gel of duck eggshell extract (*anas platyrhynchos domesticus*) with consentration 20% and 40% in vitro

Prima Agusmawanti, Mohammad Husnun Niam**, Galuh Eka Sasanti****

** Department of Pedodontics, Faculty of Dentistry, Sultan Agung Islamic University*
*** Department of Oral Public Health, Faculty of Dentistry, Sultan Agung Islamic University*
**** Dentistry Education Program, Faculty of Dentistry, Sultan Agung Islamic University*

Correspondence :primaagus@unissula.ac.id

Received 23 February 2022; 1st revision 12 October 2022; 2nd revision 15 November 2022; Accepted 21 Desember 2022; Published online 28 December 2022

Keywords:

Duck Egg Shell; Remineralization; Hardness of Primary Teeth Enamel

ABSTRACT

Background : *Children are a population that has a high potential to be exposed to caries. Calcium deficiency due to demineralization of the enamel can cause a decrease in hardness during caries pathway. It can be anticipated with the application of re-mineralizing materials. Duck eggshells are contain high amount of calcium. This research aimed to examine the effect of duck eggshell extract gel (Anas plathyrhynchos domesticus) on the enamel hardness of primary teeth.*

Methods : *This research is an experimental laboratory type using a pre and post-test control group design. There were 3 treatment groups with 4 samples in each group, which consisted of application of duck eggshell extract gel (Anas plathyrhynchos domesticus) with a concentration of 20% and 40%, and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste. Sample application was carried out 42 times in 14-day intervals. Then the sample was measured with vickers microhardness tester. The data were processed by using Paired T-Test, Kruskal-Wallis, and Post Hoc Mann-Whitney statistical tests.*

Results : *showed that there were significant differences in the samples before and after the application of duck eggshell extract gel (Anas plathyrhynchos domesticus) ($p<0.05$). Significant differences were also found in the three groups ($p<0.05$). The group that experienced the highest increase in hardness was the 20% duck eggshell extract gel (Anas plathyrhynchos domesticus) treatment group.*

Conclusion *: was that duck eggshell extract gel (Anas plathyrhynchos domesticus) with a concentration of 20% was effective in increasing the hardness of primary tooth enamel.*

doi: http://dx.doi.org/10.30659/odj.9.2.266-272
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Odonto : Dental Journal accredited as Sinta 2 Journal (https://sinta.kemdikbud.go.id/journals/profile/3200)
How to Cite: Agusmawanti *et al*. Hardness analysis of remineralization primary teeth enamel after the application gel of duck eggshell extract (anas platyrhynchos domesticus) with consentration 20% and 40% in vitro. Odonto: Dental Journal, v.9, n.2, p.266-272, December 2022

INTRODUCTION

Enamel is one of the hard structures that make up teeth consisting of 96% inorganic matter, 4% organic matter, water, and fibrous tissue, making it the hardest constituent structure on the teeth. The inorganic content is dominated by calcium, phosphate, as well as HA ions (Ca10(PO4)6 (OH)2) or FA (Ca10(PO4)6 F2. The structure of deciduous teeth is not as thick and dense as permanent tooth enamel 1 The degree of mineralization of deciduous teeth is only around 80.6% compared to the mineralization of permanent teeth which can reach 89.7%, this makes the enamel prism in the firstborn tooth not as much as the enamel prism in permanent teeth, and as a result, hydroxyapatite crystals that make up the enamel of deciduous teeth do not have a higher density than permanent teeth 2.

The presence of bacterial activity derived from food waste in the teeth causes carbohydrate metabolism and produces a type of organic acid including hydrogen ions, resulting in a drastic decrease in pH 3. This low pH of the oral cavity will trigger the occurrence of demineralization 4. The demineralization process will trigger the decomposition of calcium and phosphate ions in hydroxyapatite crystals, if this process occurs continuously it will cause microporosity in the teeth 4,5. The onset of microporosity will later have an impact on changes in the structure of tooth enamel which has an impact on reducing the hardness of enamel 6.

Restoration of enamel hardness can be achieved by remineralization which occurs if the pH of the saliva is in neutral conditions (6.0-6.8) and there are calcium ions (Ca2+) and phosphates (PO43-) with an adequate amount. The dissolution of apatite becomes normal again with the help of buffering, prevention of dissolution, as well as gaping of the dissolved ionic structure 7. Enamel that is hypo mineralized will bind calcium and phosphate ions to replace the released ions and reconstitute hydroxyapatite crystals, resulting in the hardness of the tooth enamel may increase8,9. Therefore, remineralizing agents in the form of calcium, phosphate, and fluorine are needed to improve the condition of the hypo mineralized teeth 10 There are many remineralizing agents which can be used to support the repair of mineral ions, one of which is casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) as a substitute source for loose enamel ions 11. CPP-ACP is a substance formed from dairy products, when the substance is ingested by a combination of pepsin, trypsin, and chymotrypsin enzymes as bond-breaking enzymes peptide, it will produce casein phosphopeptide which then converts calcium phosphate into a substance that can localize calcium and phosphate ions in the teeth 10,12,13,14. In addition to using conventional remineralizing agents, natural materials also have the potential to be used, for example, eggshells which in this case have the potential to produce substances useful for the process 15.

CPP-ACP is a type of remineralizing material composed of casein in the form of CPP and has a high calcium and phosphate content 16. CPP-ACP has been shown to inhibit dental demineralization and trigger remineralization, besides that CPP-ACP can also maximize fluoride activity. In assisting the remineralization process, CPP will stabilize calcium and phosphate ions, CPP-ACP is also anti-caries 10. In a study conducted by Tamara 17 and Rachmawati 16 on the effect of using the substance on teeth, it was found that an increase in enamel hardness after the use of CPP-ACP topically. The use of this substance is considered safe due to its low cytotoxic level, which is evidenced by laboratory tests in mouse fibroblast cultures that revealed that

cell tolerance after exposure to ACP- CPP ranges over 70% 18

The eggshell contains 94% calcium carbonate, 1% magnesium carbonate, and 1% calcium phosphate 19,20. Duck eggshells have a higher calcium content than other poultry eggshells 21. The increase in egg consumption in Indonesia is relatively high because eggs are a source of protein that is cheap, the amount of consumption continues to increase following Badan Pusat Statistik(BPS) data (2019) which states that per capita egg consumption per week was 2.12% in 2017 and increased to 2.15% weekly. Eggshells are organic waste that is not well utilized even though their use is always increasing and their potential is high as a source of calcium carbonate (CaCO3) 22. Duck eggshells that have been processed by calcination have a higher level of CaO purity than chicken eggshells 23.

In a previous study by Saveria 24 the use of gel extract based on duck eggshell with a concentration of 31% was shown to increase the average enamel hardness by 42.1 Vickers Hardness Number (VHN), which became a parameter that duck eggshells can be an alternative material remineralizing agent on the teeth. In this case, the author wants to make a gel material based on duck eggshell with a concentration of 20% and 40% which will be applied to the firstborn teeth as a remineralizing agent.

The ability of duck eggs to help the occurrence of remineralization in enamel has been proven by several studies, including research by Asmawati 25 succeeded in proving that the content in the duck eggshell can help increase calcium and phosphorus levels so that they can increase the hardness of enamel.

The following study aims to determine the effect of the application of duck egg shell extract gel (Anas platyrhynchos domesticus) 20% and 40% on the hardness of deciduous tooth enamel.

METHOD

The research conducted was of the experimental laboratory type using Pre-Test Post-Test Control Group Design. The sample consists of the teeth primary incisive of the upper jaw Based on the calculation results, 4 samples were used in each group. In his study, the subjects were divided intocontrol group and treatment groups. Group 1: gel application of duck eggshell extract (Anas platyrhynchos domesticus) 20%. Group 2: application of duck eggshell extract gel (Anas platyrhynchos domesticus) 40%, As well as the controls group CPP-ACP. This means that the total sample used is 12 samples.

The research began with the manufacture of duck egg shell flour and the manufacture of duck eggshell gel. Group 1 is the group that will be given the gel application of duck egg shell extract (Anas platyrhynchos domesticus) 20%, group 2 is the group that will be given gel application of duck eggshell extract (Anas platyrhynchos domesticus) 40%, and group 3 was the control group given the CPP-ACP application. It then tests the hardness of tooth enamel with the Vickers Microhardness Tester HMV M3. Furthermore, the remineralization step is carried out, the storage of the sample in a container that has been filled with artificial saliva in an incubator with a temperature of 370 C until the time of the next treatment and performed a hardness test of tooth enamel with the Vickers Microhardness Tester HMV M3.

RESULT

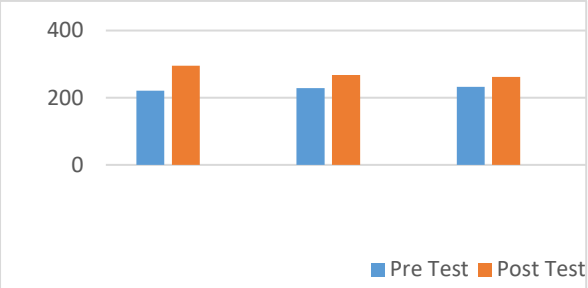

Figure 1 Diagram of the average hardness values of deciduous tooth enamel before and after treatment.

Table 2 Table of *Paired T-Test* test results

Specimens of Sig.	
Group 1 (before-after)	0.003
Group 2 (before-after)	0.001
Group 3 (before-after)	0.010

Based on the results in Table 2, it is known that there are significant differences before and after the application of duck eggshell extract gel concentrations of 20%, 40%, and CPP-ACP paste ($p<0.05$). Furthermore, to determine the effect of applying duck egg shell extract gel with a concentration of 20%, 40%, and CPP-ACP paste on the hardness of deciduous tooth enamel, further testing was carried out starting with the Shapiro-Wilk test to test the normality of each group's data.

Table 3. Table of Shapiro-Wilk test results

Specimens	Normality test Sig.
Group 1	0.019
Group 2	0.171
Group 3	0.742

Reviewing the normality test data from the table, it can be seen that the data of group 1 are not normally distributed ($p<0.05$), while the data in groups 2 and 3 are normally distributed ($p>0.05$). Following these results, the Kruskal-Wallis test was then carried out to determine the effect of the application of duck eggshell extract gel with a concentration of 20% and 40%, as well as CPP-ACP paste against the hardness of tooth enamel.

Table 4. Table of Kruskal-wallis test results

	Kruskal-Wallis Test
Asymp. Sig.	0.022

Following Table 4, it was found that there was a significant difference in the average hardness of deciduous tooth enamel in the gel treatment groupof duck egg shell extract concentration of 20% and 40%, as well as the CPP-ACP control group. It is concluded so because the value of significance that appears is 0.022 ($p\leq0.05$). Next, a follow-up test was carried out to determine the comparison of the effect of treatment on intergroups using the Mann-Whitney Post-Hoc test.

Table 5. 5 Mann-Whitney Post-Hoc test results table

Group	Mann-Whitney Test	Significancy
Group 1	Group 2	1,000
	Group 3	0.000
Group 2	Group 1	1,000
	Group 3	3,000
Group 3	Group 1	0.000
	Group 2	3,000

Table 5 shows that in the results of group 1 compared to group 2 there was no significant difference ($p>0.05$). This means that the application of duck egg shell extract gel concentration of 20% and duck eggshell extract gel concentration of 40% on the enamel of deciduous teeth does not cause a meaningful difference. Furthermore, in the results of the group 1 test compared to group 3, a significant difference was found ($p<0.05$), in other words, the application of 20% duck eggshell extract gel and CPP-ACP paste on enamel deciduous teeth make meaningful differences. Furthermore, in the last comparison, in the results of group 2 compared with group 3, an insignificant difference was obtained ($p<0.05$), it can be concluded that between the application of gels with 40% duck eggshell

extract and CPP-ACP paste on deciduous tooth enamel do not cause any meaningful differences.

Through figure 1, it can be seen that in group 1 there was an increase in hardness after the application of the treatment material, which originally had an average hardness of 220,780±6,560 VHN rose to 294,750± 13,671 VHN. Furthermore, in group 2 there was also an increase in average hardness from 228,392±11,415 VHN to 267,540±9,261 VHN. Then the last one in group 3, there was also an increase in average hardness from 232.158±7.9140 VHN to 261.941±13.472 VHN. From these results, it is known that group 1 experienced a higher increase in enamel hardness compared to the other groups.

DISCUSSION

From measuring the hardness value of enamel with the Vickers Microhardness Tester tool after day 14 of the treatment period in the entire group, there was an increase in the value of hardness in all groups. Group 1 with the review of the test material in the form of duck egg shell extract gel concentration of 20% had an average increase in enamel surface hardness of 73.97 VHN. Group 2 with a gel review of duck egg shell extract concentration of 40% experienced an increase in enamel surface hardness of 39.15 VHN. Group 3 with the application of positive control in the form of Pasta CPP-ACP had an average increase of 29.78 VHN.

Then from the results of the Paired T-test analysis, it was found that there were significant differences before and after the review of duck eggshell extract gel concentrations of 20%, 40%, and CPP-ACP Paste ($p<0.05$). Referring to these results, it was concluded that there was a significant increase in the surface hardness of the enamel of deciduous teeth. These results are following previous studies that explained that the remineralization process in enamel is very dependent on the availability of inorganic materials, especially calcium (Ca) ions 26,27.

The results of the analysis in Table 4 Kruskal-Wallis showed that the value of changes in the hardness of the enamel surface of the three groups experienced a meaningful difference with a significance value of 0.022 ($p<0.05$). These results prove that the application of duck eggshell extract gel with a concentration of 20%, 40%, and CPP-ACP paste can help the occurrence of remineralization in the enamel of deciduous teeth.

The test continued using the Mann-Whitney analysis test, the results found that no significant differences were found ($p>0.05$) between group 1 (duck eggshell extract gel concentration 20%) with group 2 (duck egg shell extract gel concentration 40%). However, when viewed from the average hardness value results, the enamel that has been applied to duck eggshell extract gel with a concentration of 20% has a higher hardnes than concentration of 40%. This condition is caused by a lower 20% concentration gel viscosity so that the dispersion power becomes better and allows attachment between the material and the enamel surface which maximum and optimal absorption of remineralizing materials 9. Previous research has proven that the composition of CMC-Na gelling agents that are less than the extract materials will cause stretches between CMC-Na molecules which triggers an increase in viscosity gels thereby lowering dispersion and attachment ability 28. Insignificant differences ($p>0.05$) also appeared between group 2 (shell extract gels) duck eggs concentration 40%) with group 3 (CPP-ACP paste). However, when viewed from the average hardness value of enamel, the hardness of enamel reviewed with duck egg shell extract gel concentration is 40% higher than the

hardness of the enamel to which the CPP-ACP paste is applied. This is still related to the interrelationship of the remineralization effectiveness of the CPP-ACP paste which depends on time. In this study, the duration of treatment was equalized between groups and one another, even though it took a duration of at least 30 minutes of application to represent the recommendations of the factory that instructed the patient to avoid eating and drinking activities for approximately up to 30 minutes after clinical use of CPP-ACP paste 27. But despite this, the duration of application of CPP-ACP paste for 5 minutes has also been used in similar studies and has been shown to be able to increase the hardness of enamel 29.

Meanwhile, significant differences ($p<0.05$) appeared between group 1 (duck eggshell extract gel concentration 20%) and group 3 (CPP-ACP paste). This happens because besides the application time of CPP-ACP paste is too short, the 20% concentration gel has a higher diffusion power compared to CPP-ACP paste. This is in line with the statement that a low viscosity will facilitate the diffusion of ions to penetrate enamel 30,31.

CONCLUSION

Based on the results of the study on the effect of the application of duck eggshell extract gel concentrations of 20%, 40%, as well as CPP-ACP paste, it can be concluded that duck eggshell extract gel (Anas platyrhynchos) domesticus) concentrations of 20%, and 40%, as well as CPP-ACP, paste as remineralizing agents influence increasing the hardness of deciduous tooth enamel. Duck eggshell extract gel (Anas platyrhynchos domesticus) concentration of 20% has the highest average value of enamel hardness increase compared to 40% concentration gel and CPP-ACP paste.

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