

Effectiveness Of Paste Based Moringa Oleifera To Increase Calcium Levels Human Tooth (In Vitro)

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Objective: To investigate of potential of moringa oleifera paste as a new material for remineralization. Material and Methods: Total of 27 samples was collected and divided into three groups (9 samples each). Group 1 as pretest data, group 2 application of paste based moringa moringaoleifera, and group 3 application of calcium phosphopeptide amorphous phosphate (cpp-acp) which was further analyzed for calcium content using atomic absorption spectrophotometry (AAS). Then all groups analyzed by using Pre-test and Post-test with Control Group Design. Results: The research results in the average rate of calcium has showed paste based Moringa Oleifera has higher calcium level compared to Pre-test groups and the CPP-ACP group. However, based on the one way ANOVA statistical test did not show significant value because ($P > 0.05$). Conclusion: with the in vitro condition choosen, paste based moringa oleifera effective increasing calcium levels of human teeth than the Pre-test and cpp-acp group even though in insignificant amounts.

Keywords: Calcium of tooth, Tooth remineralization, Moringa leaf, CPP-ACP

INTRODUCTION

Dental caries is a disease that often occurs in the oral cavity. According to Riskesdas, the percentage of population in Indonesia with oral and dental problems in 2007 and 2013 increased from 23.2% to 25.9%, which means that the tooth decay of the Indonesian population was 460 teeth per 100 people. These data indicate an increase in the prevalence of active caries in the population in Indonesia.¹

Caries is a multifactorial disease characterized by an imbalance in the process of dynamic demineralization and remineralization, which causes tooth mineral loss over time. Demineralization is the process of losing some or all of the minerals from the hydroxyapatite (HA) crystals of hard tooth tissue. One of the demineralization factors is that individuals consume acidic foods and drinks for a long period of time which causes teeth to lose HA minerals. The process of demineralization in the long term can lead to teeth more sensitive to heat, cold, pressure, pain that leads to white spot lesions.^{2,3,4}

White spot lesions can experience remineralization naturally, saliva as a key factor in helping the process of natural remineralization, is the main source of calcium (Ca^{2+}) and phosphate (PO_4^{3-}) to help super-saturation of tooth minerals that dissolve due to demineralization, certainly with a neutral pH condition of the oral cavity so that it can inhibit tooth demineralization. In order to achieve remineralization, a significant amount of calcium or fluoride is required. The calcium and phosphate content in saliva must be supported by a neutral pH condition of the oral cavity, but when the acidic conditions in the oral cavity are greater, and then natural remineralization itself is inadequate. Fluoride therapy is required to help the remineralization process. The most effective caries prevention method is through the use of topical fluoride. Calcium in hydroxyapatite is assisted by fluorine, forming apatite flour which has a much lower solubility than hydroxyapatite.^{4,5}

The ideal remineralization requirements are that it can release calcium and phosphate ions, prevent calculus formation, and work well under hypo salivation conditions as well as in acidic environments. One ingredient that is often used to assist the remineralization process is CPP-ACP, a Nano-complex that is derived from milk protein and can stabilize and increase the concentration of calcium and phosphate in saliva.⁶

Moringa leaf is a plant that has high calcium levels. The high nutritional value, efficacy and benefits have made Moringa a nickname as Mother's Best Friend and Miracle Tree. In Indonesia, it is generally considered as one type of vegetable for consumption. Moringa leaf is one part of the Moringa plant that has been widely studied in terms of its nutrition and usefulness, including calcium, iron, protein, vitamin A, vitamin B and vitamin C. Moringa leaf contains more iron than other vegetables. Moringa leaf also contains various kinds of amino acids. Calcium contained in Moringa leaf is equivalent to calcium in four cups of milk. In addition, Moringa leaf is also identified to contain antioxidants and antimicrobials which are quite high.^{7,8}

Based on the description in the background, the researcher is interested in conducting research on Moringa leaf, because Moringa leaf contains high enough calcium so that it is expected to be one of the natural ingredients used in increasing calcium levels of teeth by using post extraction premolar teeth as samples.

MATERIALS AND METHODS

This research used the Experimental Laboratory method, namely testing conducted in a laboratory in the form of Pre-Post-Test with Control Group Design. The type of this research is a True Experimental Laboratory. Sampling method with Purposive Sampling used 3 groups. The statistical test used One Way Anova. The subjects in this study were moringa-based paste (*Moringa Oleifera*) and CPP-ACP (GC, Japan). The object of this research was premolar crowns which were measured in calcium using an atomic absorption spectrophotometer (AAS).

Samples of premolar crowns were cut mesio-distally in 2 parts (palatal and buccal). The palatal / lingual part is divided into 2 parts so that 3 pieces of teeth are obtained. The first part of the tooth was used as the Pre-test group (without treatment), the second part as a group 2 (the treatment with Moringa leaf calcium extract of paste preparations), and the third as a group 3 (treatment with CPP-ACP application). Each sample was applied to 1 ml of Moringa leaf paste and CPP-ACP using a spoon and then flattened to the entire surface of the sample using a cotton bud for 3 minutes then allowed to stand for 30 minutes, then the sample was rinsed using aquadest and dried. Application was carried out in the morning and evening. The treatments were given routinely for 7 days to get the maximum effect.

Each tooth sample was weighed on parchment paper and then measured using a digital scale and noted the weight of each tooth sample. Furthermore, all samples were put into test tubes that have been labeled according to the numbering of samples and then put into a fume hood. Each sample was dissolved using the wet destruction method to avoid mineral reduction due to evaporation using 3ml of concentrated HNO₃ as a solvent then the sample was allowed to stand for 2x24 hours until the dental sample dissolved.

Next, the first dilution was carried out up to 20 ml by adding aquadest to each sample, homogenized and then transferred into plastic bottles that have been numbered on the label. Then a second dilution is carried out by adding 1 ml of the calcium solution of the sample and transferred into a 25 ml volumetric flask and then adding distilled water to sufficient 25 ml and homogenized. Furthermore, the absorbed samples were measured using an atomic absorption spectrophotometer (AAS) at a wavelength of 422.67 nm. Absorbance was recorded and calcium levels found in the teeth was calculated.

To obtain calcium levels from measurements using the atomic absorption spectrophotometer, the formula was used:

$$Ca = \frac{(\text{conc}) \times \text{Vol} \times \text{fp}}{\text{sample weight}}$$

Information :

Ca = calcium levels obtained from the measurement results ($\mu\text{g} / \text{g}$)

Conc = instrument concentration ($\mu\text{g} / \text{ml}$)

fp = dilution factor

Vol = volume of sample solution (ml)

Sample Weight (g)

RESULTS

This research was conducted in two laboratories, the Pharmacy Laboratory of the Faculty of Pharmacy, Muslim University of Indonesia and the Makassar Health Laboratory Center for 1 month from 26 December 2019 to 21 January 2020.

There were 9 samples of intact permanent premolar teeth after extraction. Then those 9 teeth were divided into 3 parts using a mini Grinder cutting machine. So that the total sample were 27 and divided into 3 different treatment groups. In the first group, samples were measured as preliminary data and no treatment was given. The second group was given treatment in the form of the application of Moringaleaf(*MoringaOleifera*) paste material. The third group was given treatment in the form of the application of CPP-ACP material. In the final stage, the calcium level of the sample was measured using an atomic absorption spectrophotometer with a wavelength of 422.67 nm to see the absorption or absorbance of the sample.

All subsequent research results were collected and recorded, and data processing and analysis were carried out using the SPSS version 25 program (SPSS Inc., Chicago, IL, USA). The results of the research are displayed in the distribution table as follows.

Table 1. Average Calcium Levels of Teeth in the Pretest and Post Test Groups (Application of Moringa LeafPaste and CPP-ACP Application)

Group	Calcium levels ($\mu\text{g}/\text{gr}$)	
	N	Mean(SD)
Pre test(I)	9	276563±64823.05
Moringa LeafPaste (II)	9	366091.11±150918.87
CPP- ACP (III)	9	348297±83507.73

Based on the average test results in Table 1, it can be seen that the Moringaleaf paste group has the highest average calcium level compared to the pre-test group and the CPP-ACP group, while the pretest group has the lowest average calcium content among the CPP-ACP group and Moringaleaf paste group. Next,both the Kolmogorov-Smirnov normality test and the Shapiro-Wilk normality test all showed $p > 0.05$. Data from this

research can be stated to have normal distribution. So that it can be continued for One Way ANOVA test.

Table 2. Differences in Average Calcium Levels of Teeth in the Pretest Group, Application of Moringa Leaf Paste and CPP-ACP Application

Group	Comparison	Mean Difference	Std. Error	p-value/sig.	p-value ANOVA
Pretest (without application)	Moringa Leaf	-89528,11	50149,4	0,087	0,189
	CPP- ACP	-71734,00	50149,4	0,165	
Moringa Leaf	CPP- ACP	17794,11	50149,4	0,726	
*. The mean difference is significant at the 0.05 level.					
*Post Hoc test: Low Significant Difference (LSD) test; $p < 0.05$: significant					

Based on research conducted, Table 2 shows that there are differences in the average levels of dental calcium in the pre-test and post-test groups (Moringaleaf paste and CPP-ACP). In the comparison test of the average calcium level in group I with the calcium level in group II showed a p-value of 0.087 ($P > 0.05$), there is a difference in the average value (Mean Difference) that is equal to -89528.11. This result means that the calcium level in group II (Moringa leaf paste) is greater than the average calcium level in group I (Pre-test).

Furthermore, in the comparison test of the average calcium level in group I with the calcium level in group III showed a p-value of 0.165 ($P > 0.05$), there is a difference in the average value (Mean Difference) that is equal to -71734.00. This indicates that the calcium level in group III (CPP-ACP) is greater than the average calcium level in group I (Pre-test).

Then the comparison of average calcium levels in group II with calcium levels in group III shows a p-value of 0.726 ($P > 0.05$), there is a difference in the mean value (Mean Difference) that is equal to 17794.11 in the calcium level of this group. It is the average calcium level in group II (Moringaleaf paste) is greater than the calcium levels in group III (CPP-ACP).

However, based on the One Way ANOVA test results, the p-value showed $p = 0.189$ ($P > 0.05$). This means that there are insignificant differences in dental calcium levels in the pre-test and post-test groups (Moringa Leaf Paste and CPP-ACP).

DISCUSSION

This research was conducted to prove that the paste made from Moringa leaf (Moringa Oleifera) as a natural ingredient which contains calcium mineral content in a

high amount has an effect in increasing calcium levels of human teeth. Table 2 shows that there were differences in the average levels of dental calcium in the pre-test and post-test groups (Moringaleaf paste and CPP-ACP). Although statistically this research showed that an increase in calcium levels in the Moringa leaf paste group was less significant in increasing calcium levels in human teeth, but the average calcium level in the Moringa leaf paste group was higher than in the CPP-ACP group. This might be due to the fact that Moringa leaf has a very high calcium level so that it can increase the calcium level in teeth equivalent to CPP-ACP.

In line with Khalaf's research, E et al (2016) said that Moringa leaf extract (*Moringa Oleifera*) has the same remineralization effect of enamel and dentin as CPP-ACPF paste in preventing erosion from enamel and dentin by localizing calcium and phosphate on tooth surface. In SEM (Scanning Electron Microscope) analysis, enamel specimens of uremic patients after Moringa leaf extract was applied, it is occurring the closure of the enamel prism by the appearance of calcium mineral deposits along the porous and a decrease in the diameter of the dentinal tubules which is greater than CPP-ACPF.⁹

In Aminah's research (2015) it was found that Moringa leaf has many mineral contents, it contains mineral content of phosphorus around 0.004% and calcium in a high amount equivalent to calcium in 4 cups of milk in which the mineral content can be used as a mineral substitute for demineralized dental minerals.⁸

However, the research that has been conducted did not reach a significant value, so it can be said this research still needs some improvement. Moringa leaf paste can be added flour to help the process of remineralization of human teeth. In line with research González (2018) said that remineralization of enamel requires calcium and phosphate ions to be able to diffuse into the porous sub surface of the enamel through the intact surface zone. Remineralization can occur or be induced through therapy, including therapies available that is fluoride-based treatment. Calcium and phosphate are important minerals for remineralization.¹⁰

In this case, the CPP-ACP's lack of effectiveness in increasing calcium levels may occur due to several factors, one of which is the length of application and the condition of the oral cavity and neutral salivary pH. This research was conducted in vitro without directly involving the oral cavity and saliva while CPP-ACP works better with saliva assistance causing the value of calcium levels obtained from the CPP-ACP group to be lower than the Moringa leaf group.

In line with research conducted by Widyasari and Puguhbayu (2012) proved that the use of CPP-ACP can play a role in the prevention of oral caries. The average level of calcium in the enamel group that was given CPP-ACP application for 28 days was the highest compared to the group that was given CPP-ACP for 3 and 14 days while in this research

was only done for 7 days, also in the instruction for the use of CPP -ACP is recommended not to be rinsed and better merged by itself with saliva, but this research was conducted in vitro so it did not involve salivary contamination.^{11,12}

Remineralization is defined as the process of redeposited calcium and phosphate ions from the environment into demineralized enamel crystals. The remineralization process can occur if the pH is neutralized and there are sufficient quantities of Ca^{2+} and PO_4^{3-} ions. Casein phosphopeptide - ACP (CPP-ACP) has been developed for remineralization purposes. CPP aggregation with calcium phosphate forms the ACP group. This aggregation prevents the deposition of calcium phosphate and hence produces a super-saturated state in connection to enamel, thus preventing demineralization and increasing remineralization.^{13,4}

From the results of the research in table 5.3 shows that there is no significant difference in the calcium content in teeth before and after the application of Moringa leaf (*Moringa Oleifera*) with CPP-ACP application. Although it did not increase significantly, the calcium content of Moringa leaf was still higher on average compared to CPP-ACP. Moringa leaves are beneficial to human health, high enough nutritional content makes Moringa as a food supplement, as a source of natural antioxidants, anti-inflammatory, antibacterial and even anti-cancer.

In line with research conducted by Su-Kyung Jwa (2019) who tested the preventive effects of Moringa leaf on biofilm caries induction. Moringa leaf mostly contains protein, followed by fatty acids and phenolic molecules. The results of the research reported that Moringa leaf extract has antimicrobial activity toward cariogenic bacteria and biofilms. Caries is caused by bacterial interactions which are mostly *Streptococcus mutans*. Bacteria break down fermentable carbohydrates such as glucose, sucrose, and fructose and cause an acidic environment that leads to tooth demineralization. Thus, this research was conducted to find out how effective Moringa leaf is in increasing dental calcium which can be developed into natural remineralization ingredients.^{14,8,15}

CONCLUSION

Based on the results of the study, it can be concluded that there is an increase in the amount of calcium in the teeth after the application of Moringa oleifera based paste and CPP-ACP application. Moringa oleifera based paste and CPP-APP are effective to increasing calcium levels in teeth compared to the pre test group. However, statistically in this study there were no significant differences regarding the effectiveness of Moringa oleifera based paste and CPP-ACP to increasing dental calcium levels. Moringa oleifera based paste can also be developed as an additional ingredient in remineralization or tooth calcification.

SUGGESTION

Further research can be made of paste made from *Moringa oleifera* as tooth remineralization materials with more attention to the active compounds of *Moringa oleifera* leaf, isolation method to get pure calcium content from *Moringa* leaves and the addition of supporting materials such as flour to be used as dental remineralization. Further tests need to be carried out to see the consistency, water absorption, water content, thermoresistance and particle size in the paste made from *Moringa* leaves. Further tests such as in vivo testing and toxicity tests need to be carried out so that the paste made from *moringa oleifera* leaves can be maximally utilized.

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