ORIGINAL PAPER

ASSESSMENT OF ENAMEL RE-MINERALIZATION OF DECIDUOUS TEETH AFTER TREATMENT WITH *CURCUMA LONGA* LINN. AND CASEIN PHOSPHOPEPTIDE-AMORPHOUS CALCIUM PHOSPHATE FLUORIDE

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ABSTRACT

Introduction: *Curcuma longa* Linn. (CLL) has high calcium and phosphate concentrations, resulting in increasing re-mineralization of enamel.

Objectives: This study was to determine the difference between CLL and casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF) in the growth of enamel re-mineralization.

Material and methods: CLL 5% and 10% were obtained from thick CLL extract and weighed to acquire 50 grams of CLL extract. 2.5 grams of CLL were taken, and 2.5 grams of carboxymethyl cellulose (CMC) and 45 ml of distilled water were added, giving CLL 5% gel. For CLL 10% gel, 5 grams of CLL, 2.5 grams of CMC, and 42.5 ml of distilled water were mixed. De-mineralization solution consisted of 2.2 mM/l $CaCl_2$, 2.2 mM/l KH_2PO_4, and 50 mM acetate buffer, with pH of 4.06. Samples of 24 extracted primary maxillary central incisors were divided into four groups: 1. Negative control group (C–); 2. Positive control (C+) with CPP-ACPF 5% cohort; 3. CLL 5%; and 4. CLL 10% treatment group.

Results: Mean enamel micro-hardness in CLL 10% group after re-mineralization at tenth minute time-point (449.28 HV) was higher than that in CPP-ACPF 5% group (403.41 HV), which was significantly different. Strongest correlation coefficient was observed in CLL 10% group ($r = 0.820$, very strong), with highest R2 value of 67.2%.

Conclusions: CLL 10% is more effective than CLL 5%. Moreover, CPP-ACPF 5% was found efficient in increasing enamel re-mineralization, as a potential agent for re-mineralizing primary teeth enamels.

Key words: hardness, curcuma, enamel, tooth re-mineralization.

J Stoma 2022; 75, 1: 19-24
DOI: https://doi.org/10.5114/jos.2021.112039

INTRODUCTION

In Indonesia, 73.4% of children between 10-years and 14-years experience cavities, therefore dental caries remains a problem in this age group [1]. In school-age children, caries rate is show as high as 60-90%, and in adults, it reaches nearly 100%, causing pain and discomfort [2]. Efforts to prevent the occurrence of caries is to prevent the release of minerals in teeth and their de-mineralization, by improving the process of re-mineralization. A material widely used in dental practice to inhibit enamel de-mineralization is casein phosphopeptide-amorphous calcium

JOURNAL OF STOMATOLOGY
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Received: 18.05.2021 • Accepted: 02.08.2021 • Published: 20.12.2021

phosphate (CPP-ACP). It has a significant contribution to reduce the development of carious lesions on the enamel, and has a significant additive effect that slows de-mineralization process and promotes prebiosis [3-5]. In addition to CPP-ACP, there is casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), which aims to improve the process of re-mineralization and to increase tooth resistance to acid, by forming mineral deposits of calcium and phosphate ions with fluorine [6]. Re-mineralization in teeth requires calcium, phosphate, and fluoride [7]. Phosphate is a particle that contains phosphorus, which is needed in the teeth and bones.

Nowadays, modern science is starting to recognize and understand the extraordinary healing qualities of turmeric (*Curcuma longa* Linn., CLL), and many studies are currently underway to prove its efficacy in systemic and dental health [8]. CLL contains calcium, phosphate, and antibacterial properties [9]. Calcium and phosphate in CLL is deposited on a surface layer of micro-porosity, and then enter porosity of primary tooth enamel [10].

OBJECTIVES

This study was conducted to determine the difference between CLL and CPP-ACPF in increasing enamel re-mineralization due to previous de-mineralization. Research hypothesis was that CLL and CPP-ACPF would have a different effect on micro-hardness of primary maxillary central incisors with previous enamel de-mineralization.

MATERIAL AND METHODS

RESEARCH DESIGN AND SAMPLE

This study was entirely a laboratory experiment, using a pre-test/post-test control groups design. The samples used were primary maxillary central incisors, which have been extracted due to persistence, and included caries-free teeth, with no fracture, no restoration, and no anomaly. To determine the sample size, Federer formula, i.e., $(t - 1)(n - 1) \geq 15$, was applied [11]. There were 4 study groups, with 6 samples in each group, consisting of untreated negative control group, positive control group with CPP-ACPF 5% (GC Tooth Mouse), treatment group 1 with CLL 5% gel, and treatment group 2 with CLL 10% gel. The total sample group were 24 primary maxillary central incisors.

MAKING THE EXTRACT AND GEL OF *CURCUMA LONGA* LINN.

150 grams of CLL powder was obtained by washing and peeling CCL skin, and then placing it in oven for 2 hours at 60°C. After that, it was milled in a blender and sieved with a 200-mesh sieve to obtain a fine CLL powder. CLL was dissolved in 1.2 liters of ethanol 96%, and an extraction was processed by maceration using ethanol 96%. The acquired extract was stored for 48 hours, then stirred using a stirrer, and was concentrated using a rotary evaporator at 50°C for 1 hour, until a thick extract was obtained; it was weighed to get 50 grams of CLL extract. From the thick CLL extract, 2.5 grams (5% of 50 grams) were taken, with 2.5 grams (5% of 50 grams) of carboxymethyl cellulose (CMC) and 45 ml of distilled water added to obtain 5% CLL gel. In order to acquire 10% CLL gel, 5 grams of thick CLL extract (10% of 50 grams) was required and combined with 2.5 grams of CMC (5% of 50 grams) and 42.5 ml of distilled water. The addition of CMC and distilled water to the thick CLL extract transformed the mixture into CLL gel.

ENAMEL HARDNESS TEST

Twenty-four primary maxillary central incisors of buccal surface were implanted in acrylic resin. Previously, the roots were cut using a low speed micromotor (Strong 207B; Micro Motor, South Korea) with a carborundum disc, until the crown was left (Figure 1). The buccal was planted into a 1 cm diameter pipe using acrylic resin. The surface of a sample was cleaned for 3-4 minutes with a brush to get a surface clean from debris. Furthermore, the 24 teeth were immersed in a de-mineralization solution of 2.2 mM/l $CaCl_2$, 2.2 mM/l KH_2PO_4, and 50 mM acetate buffer for 1 hour, with pH acidity of 4.06 [12, 13]. They were washed using a syringe and left for 5 minutes, and then, the enamel micro-hardness after application of de-mineralization solution was measured with a Vickers hardness tester (premium micro-Vickers hardness tester, EW-105 series; UK). Tip of diamond indenter was pressed against the surface of a sample, with a load of 300 g for 10 seconds. The hardness was measured at three different points in each sample, including upper, middle, and lower areas (area close to the cervical), and mean value was calculated. The sample of primary maxillary central incisors was grouped into 4 groups, using random sampling method, with each group consisting of a negative control group without treatment, positive control CPP-ACPF 5% group, treatment 1 CLL 5%, and treatment 2 CLL 10%, using a tip applicator. The four groups' samples were placed in different petri dishes openly and left for 5 and 10 minutes, with their hardness measured at 5-minute and 10-minute time-points, in similar way as the method used for measuring enamel micro-hardness. The use of de-mineralization material (pre-test) before CPP-ACPF and CLL application (post-tests) was to determine the increase in enamel micro-hardness, which was reduced due to previous de-mineralization [14].

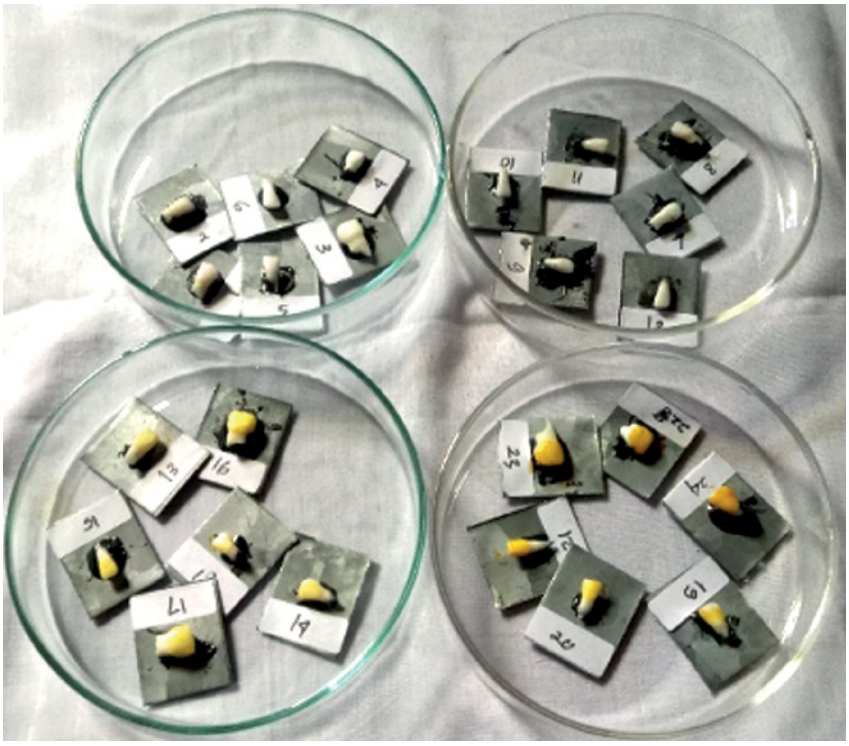


FIGURE 1. Preparation of the specimens for testing (twenty-four primary maxillary central incisors of buccal surface)

STATISTICAL ANALYSIS

Paired *t*-test was applied to evaluate the differences in enamel micro-hardness before and after re-mineralization treatments in each group. One-way ANOVA test was used to evaluate the differences between the three groups after treatment. *P*-value < 0.05 was considered statistically significant. If there was a significant difference in ANOVA test, Pearson's correlation test and linear regression were carried out to determine the contribution of length of application time to the increase of re-mineralization.

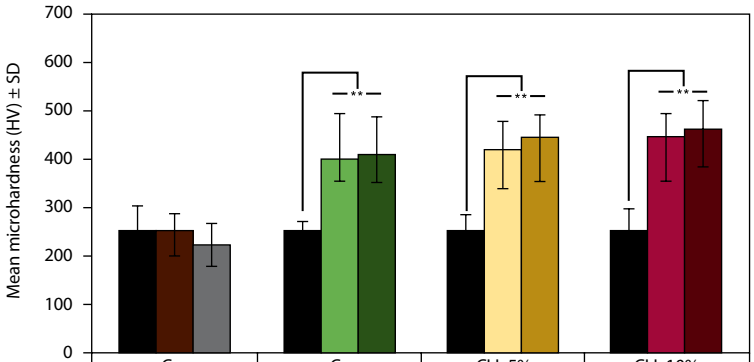
The study was approved by ethics committee of Faculty of Medicine of the University of Brawijaya, Indonesia (No. 44/EC/KEPK-S1-FKG/02/2017).

RESULTS

Paired *t*-test illustrated a significant difference in enamel micro-hardness between the de-mineralization groups, with the C+ treatment group at 5-minute time-point ($p = 0.007$) and the C+ treatment group at 10-minute time-point ($p = 0.002$). There was a significant difference at 5-minute time-point ($p = 0.001$) and 10-minute time-point ($p = 0.000$) measurements between the de-mineralization groups and the CLL 5% treatment group. Moreover, the CLL 10% treatment group presented a significant differences at 5-minute time-point ($p = 0.000$) and 10-minute time-point ($p = 0.000$) measurements compared to those of the de-mineralization groups. However, re-mineralization at 5-minute and 10-minute time-points of the C+, CLL 5%, and 10% groups were not significantly different.

The mean enamel micro-hardness after 5 minutes in the positive control (C+), CLL 5%, and CLL 10% groups were 397.00 HV, 417.55 HV, and 428.18 HV, respectively, while in the negative control (C–), it was 246.21 HV. At 10-minute time-point, the C+, CLL 5%, and CLL 10% groups presented 403.41 HV, 438.55 HV, and 449.28 HV, respectively, with the C– group of 232.56 HV. The mean enamel micro-hardness of the C– group decreased from 5th minute to 10th minute. According to one-way ANOVA test, at 5-minute time-point, there was no significant difference between the three treatment groups. However, there was a significant difference ($p = 0.000$) between the three treatment groups and the C– cohort. At 10-minute time-point, there was a significant difference between the C+, CLL 5%, and CLL 10% treatment groups, and the C– cohort ($p = 0.000$). The 10% CLL treatment group differed significantly from the C+ ($p = 0.046$). Nonetheless, the differences between the C+ and CLL 5%, and between CLL 5% and 10% were not significant (Figure 2).

Pearson's correlation test results (Table 1) showed that there was a significant positive correlation between tested material and re-mineralization time. A positive correlation was observed in CPP-ACPF 5%, CLL 5%, and CLL 10% groups, and the longer they remained on the tooth enamel, the more the re-mineralization effect increased. The highest correlation coefficient occurred



	C–	C+	CLL 5%	CLL 10%
Demineralization	246.6167 ± 41.52	241.0067 ± 20.80	243.9667 ± 59.20	242.7500 ± 40.30
Remineralization 5th minute	246.2167 ± 40.40	397.0000 ± 86.13	417.5500 ± 42.50	428.1833 ± 52.50
Remineralization 10th minute	232.5667 ± 33.30	403.4167 ± 75.30	438.5500 ± 33.80	449.2833 ± 59.80

**p < 0.05; **p < 0.01; ns = not significant; SD = standard deviation*

FIGURE 2. Mean differences in enamel micro-hardness before (de-mineralization) and after treatment (re-mineralization) with CPP-ACPF 5% (C+), CLL 5%, and CLL 10%

TABLE 1. Correlation between tested materials and re-mineralization

Material	Re-mineralization at 5-minute and 10-minute time-points	
	r	*p*
CPP-ACPF 5%	0.684 (strong)	0.002
CLL 5%	0.809 (very strong)	0.000
CLL 10%	0.820 (very strong)	0.000

in the CLL 10% treatment group (r = 0.820, p = 0.000). Linear regression was applied to determine the contribution of the length of application time to the increase of re-mineralization. The results are presented in Figure 3.

DISCUSSION

Enamel re-mineralization is very important for teeth to reduce the occurrence of dental caries. Since teeth do not have the ability to re-mineralize, additional re-mineralization agents are needed [15]. Re-mineralization is a process, in which apatite crystals are formed again on enamel, so that enamel's micro-hardness, which declines due to de-mineralization, can improve [14]. Diffusion of calcium and phosphate ions that are good for the re-mineralization process, can occur if there is a low viscosity, allowing re-mineralized gel to penetrate into enamel micro-porosity. Deposition process of calcium and phosphate would then result in closure of enamel micro-porosity, called 'enamel re-mineralization' [11, 16]. Enamel micro-porosity requires a high concentration of calcium and phosphate, which cause a rapid deposition of calcium and phosphate on enamel [17]. One of the reasons for CLL treatment (re-mineralization) is a high mean enamel micro-hardness, since CLL contains 182 grams of high calcium and 268 grams of phosphate [18, 19]. CPP-ACPF 5% has a higher re-mineralization effect than CPP-ACP. CPP-ACPF contains free calcium phosphate and active fluoride ion, which helps maintaining saturation and preventing de-mineralization [20]. The mean enamel micro-hardness in the occlusal area is around 359 to 424 VHN (HV). Meanwhile, in the cervical area, the enamel micro-hardness ranges from 227 to 342 VHN (HV). This variation is caused by different factors, such as histological features, chemical composition, specimen preparation (in case of hardness measurements taken), and load errors given in indentation length (IL) [21]. The recommended range of enamel micro-hardness is 227–424 HV [22]. In the current study, all treatment groups showed an increase in the mean enamel micro-hardness, except for the negative control group (C–), where the mean value decreased, since there was no re-mineralization treatment's agent (Figure 2). At 10th minute after the CLL 10% treatments, the mean micro-hardness was the highest, i.e., 449.38 HV, which was significantly different from CPP-ACPF 5% (403.41 HV), whereas in the CLL 5% group (438.55 HV), there was no significant difference. At 5-minute time-point, there were no significant differences within the three treatment groups, except for the negative control group (Figure 2). The high mean enamel micro-hardness in the CLL 5% and CLL 10% groups were above the recommendations because of a chemical composition of CLL, which has a high calcium and phosphate substances [17, 18, 21].

In this study, the treatments used in the CPP-ACPF 5%, CLL 5%, and CLL 10% groups presented long-term effect; the longer the application on the tooth enamel surface, the better increase of the re-mineralization effect (Table 1). The duration of CLL 10% application contributed to the 67.2% of increase in the re-mineralization compared to the C+ 5% and CLL 5% treatment

groups (Figure 3). This proves that CLL 10% has the potential to be a re-mineralization agent for de-mineralized primary teeth enamels. The CLL 10% can be used on a sub-cellular scale, with high accuracy in reaching cellular targets and obtaining maximum therapeutic effect. CLL can also inhibit cariogenic properties of *Streptococcus mutans*, proving that CLL essential oils present anti-cariogenic properties [23].

Previous studies have shown that the application of CPP-ACPF has prevented de-mineralization of enamel. In a research of Hendrawan *et al.*, it was observed that application of CPP-ACPF could prevent a significant decrease in micro-hardness of the enamel [24]. Moreover, a research of Zenouz *et al.* demonstrated that the application of CPP-ACPF could significantly increase micro-hardness of de-mineralized enamel [25]. In line with previous studies, the application of CPP-ACPF in the current study could prevent a decrease in the micro-hardness of the enamel, but the mean value of micro-hardness of the enamel, on which CLL was applied, was higher than in the group of teeth, in which CPP-ACPF used. It shows that CLL is more effective in enhancing re-mineralization of the enamel.

In order to obtain better research results, the initial micro-hardness range of a sample should be reduced, so that it becomes more homogeneous, and therefore, provide more precise results. Moreover, post-test (re-mineralization) and pre-test (de-mineralization) micro-hardness measurements should be considered to minimize bias, and to improve accuracy of results [26].

CONCLUSIONS

The results of the present study show that the CLL 10% treatment is more effective than the CPP-ACPF 5% and CLL 5% in enhancing the enamel re-mineralization of the primary maxillary central incisors. The correlation results demonstrate a positive correlation between the length of time of treatment and the increase in enamel re-mineralization. Moreover, the strongest correlation coefficient and the highest R^2 of 67.2% was observed in the CLL 10% treatment group.

ACKNOWLEDGEMENT

Thanks to the Head of Mechanical Engineering Laboratory, State University of Malang, the Head of UPT Materia Medica Batu-Malang Laboratory, and all parties who have supported this research.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

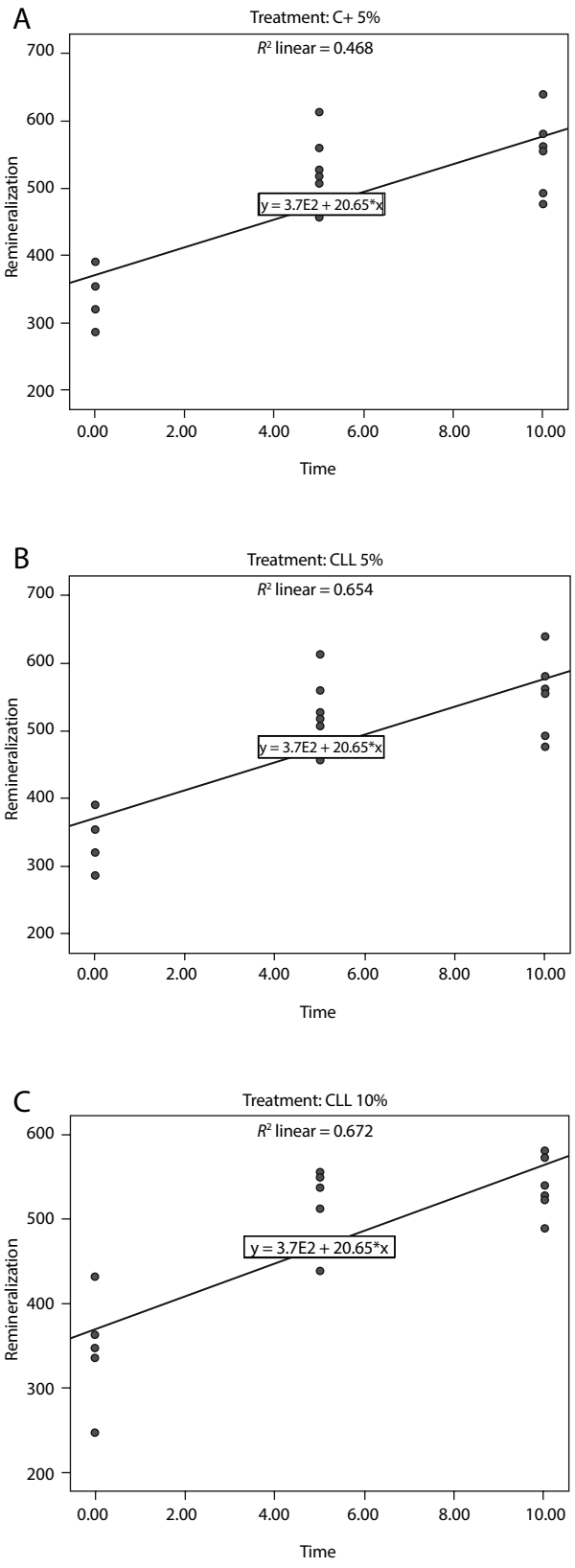


FIGURE 3. A) R^2 linier C+ 5%. **B**) R^2 linier CLL 5%. **C**) R^2 linier CLL 10%

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