The correlation of dentin elastic moduli and pH after exposed to combination of calcium hydroxide-propolis-propylene glycol

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Abstract

Previous research states that adding propolis to powdered dental materials can increase the mechanical strength of the material. To analyze the differences and correlation of dentin elastic moduli and pH value after the exposure of calcium hydroxide, a mixture of propolis and calcium hydroxide, also a mixture of propylene glycol (PG), calcium hydroxide, and propolis. The dentine of bovine incisors was exposed into various compositions of a mixture of propolis, PG, and calcium hydroxide. The measurement of pH value and dentin elastic moduli was performed after 7 days. To find difference among groups, one-way ANOVA was used, and Honestly significant difference (HSD) Tukey to compare each groups, followed by Pearson to define the correlation. A statistically meaningful difference was recorded between the groups ($P < 0.05$), and there was correlation between dentin elastic moduli and pH value. The more alkaline the environment, the more rigid the dentin.

Key words: Calcium hydroxide, dentin elastic moduli, medicine, pH, propolis, propylene glycol

INTRODUCTION

Carious lesion is the result of carbohydrates metabolism process by cariogenic bacteria which can bring down the oral pH value and further cause localized demineralization of inorganic materials and destruction of dental organic material.\(^1,2\) The demineralization process, without treatment, may develop infection and bring about dental pain. One treatment of choices for pulpitis is pulp capping, a method of applying medicament on the exposed tooth tissue, or directly on the pulp to maintain its vitality. This procedure may avoid further decay, which required more complicated and expensive treatment procedures.\(^3\)

Calcium hydroxide is one of the most common pulp capping materials which is considered as the gold standard inspite of its flaw in low compressive and tensile strength, low elastic moduli, and highly soluble.\(^4,5\) Dentin exposed to calcium hydroxide may have a slightly changes in its elastic moduli. Restoration materials are expected to have good elastic moduli, so that it can adapt on cavity margin fairly, to reduce a possibility of marginal leakage.\(^6\) The alkaline nature of calcium hydroxide is able to neutralize, dissolve, and denature the acidic proteins and proteoglycans in dentin. However, the acidic state in cavity can also

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neutralize its carboxyl group, thus, weakening the bond between hydroxyapatite crystals and collagen.\cite{7,8} The weakened bond between hydroxyapatite crystals and collagen caused by both alkaline and acidic milieu can affect the dentin elastic moduli which in turn can affect the marginal adaptation of teeth with restorative materials.\cite{9}

Previous research revealed an increase of mechanical strength of powdered dental material after supplemented with propolis.\cite{10} Thus, many researchers are interested in combining propolis with pulp capping agent to compensate its flaw. Studies found that combining propolis and calcium hydroxide can induce a better dental hard tissue formation, some others found its effect in multiple ratios.\cite{11,12}

A common carrier for calcium hydroxide, other than propolis, is propylene glycol (PG). This material can enhance the calcium hydroxide effectiveness by stimulating the long-term release of hydroxyl ions and promoting calcium hydroxide passing through the dentin tubules.\cite{13} Furthermore, by the formation of intermolecular bonds, PG can increase bond strength and reduce setting time.\cite{14}

As a result, PG was chosen in this study as a pulp capping accelerating agent by combining propolis and calcium hydroxide.

Due to the above-mentioned outcomes, this study aims to analyse the differences and correlation of dentin elastic moduli and pH value after exposed to combination of PG and propolis into calcium hydroxide.

**MATERIALS AND METHODS**

This was a laboratory-based analytical study, which focused on the difference and correlation of dentin elastic moduli and pH value after exposed to the combination of PG and propolis into calcium hydroxide. Ethical approval for this study was given by the institutional review board with number 338/HRECC. FODM/XII/2018.

A number of 27 bovine teeth were used as the sample. For pH value analysis, a number of 3 groups of samples are prepared. Group I, filled with calcium hydroxide and distilled water; Group II, filled with calcium hydroxide and propolis; Group III, filled with calcium hydroxide, PG, and propolis. Meanwhile, for dentin elastic moduli analysis, the samples were divided into 4 groups, and received the same treatment; with additional control group.

**Sample preparation**

The extracted lower bovine incisors were immersed in saline solution. An access opening was performed for each tooth and root canal preparation using k file 80 subsequently and irritated with saline solution. The root canal was then filled using lentulo and low-speed needles. The root canal fillings were made with a ratio of calcium hydroxide and distilled water into 1:1 for Group 1; a composition of 1.5:1 propolis and calcium hydroxide for Group 2; a ratio of 1:1.5:1 calcium hydroxide, PG, and propolis for Group 3. The coronal part of those teeth was then covered with a cotton pellet and filled with composite restoration. A resin composite sealing was also applied on the apical part of the teeth. The samples were then immersed in saline solution for 7 days.

**pH analysis**

First of all, we decapitated the coronal part of the teeth and made a root section at 5 mm by means of saw disc. The cut was kept in a container filled with deionized water for xx. The samples were then vibrated using ultrasonic, and the pH value was measured using pH meter.

**Dentin elastic moduli analysis**

To analyze the elasticity of the exposed dentin, the root of the teeth was made into a section of 10 mm × 3 mm × 3 mm, then embedded into an acrylic plate. Then, the samples were tested using a universal testing machine. The data were obtained and calculated using the formula:

\[
E = \frac{1}{4bh^3} \left(\frac{l}{m}\right)\]

Where \(E\) is the modulus of elasticity, \(m\) is displacement curve, \(l\) is specimen width, \(b\) is specimen length, and \(h\) is specimen height.

**Statistical analysis**

The data were shown in mean and standard deviations. Shapiro–Wilk test was conducted to determine the normality of the data distribution followed by homogeneity test using Levene Test. To analyze the differences, one-way ANOVA test was used with a significance level of 0.05 followed by the Post Hoc Tukey HSD test and correlation test using the Pearson test.

**RESULTS**

The result of the study was tabulated in mean and standard deviation, shown in Table 1 (pH value) and Table 2 (dentin elastic moduli). According to Shapiro–Wilk Test, the data had a normal distribution since the pH measurement results of all treatment groups were significant with \(P = 0.200\). According to Levene Test, the data were homogeneous with a \(P = 0.291\). While the data for dentin elastic moduli also showed a normal distribution \((P = 0.200)\) and homogeneity \((P = 0.291)\).

The result showed a significant difference in terms of pH value \((P = 0.000)\). Sample with highest pH value was recorded from Ca (OH)\(_2\) and distilled water group, which significantly higher than group exposed to combination of propolis and Ca (OH)\(_2\) \((P = 0.000)\), and combination of Ca (OH)\(_2\) propolis and glycol \((P = 0.000)\) had the lowest pH value. As for dentin elastic moduli scores, the dentin
only (control) was recorded to be the lowest compared to
the combination of Ca (OH)₂ propolis and glycol (P = 0.000),
combination of Ca (OH)₂ and propolis (P = 0.000), and a
mixture of Ca (OH)₂ and distilled water (P = 0.000).

As showed in Table 3, the correlation test showed a P > 0.01,
which means significant in all groups. Thus, the pH value
was significantly correlated to the dentin elastic moduli in
all groups.

**DISCUSSION**

Bovine teeth was chosen as the sample of this study due to
their structure are similar to human teeth, and also their
abundant existence. Study found no significant difference
in diffusion level calcium hydroxide between human and
bovine teeth.

Calcium hydroxide nature is alkaline, which can causes
necrosis as it reaches the pulp tissue, disrupting the cell
proliferation, thus decreasing cell differentiation, holding
up the formation of Type 1 collagen.[13] Thus, studies
performed to compensate its flaw by combining calcium
hydroxide with propolis, which its properties do not
interfere the calcium dissociation process.[16] However, it
takes time for the combination of calcium hydroxide and
propolis to set, makes it impractical to be used directly
under resin restoration since the resin is hydrophilic.

This may affect the resin bonding system with teeth.[14] To
overcome this limitation, PG was added to the calcium
hydroxide-propolis combination to decrease the setting
time. PG enhanced the calcium hydroxide effectiveness
by inducing the long-term release of hydroxyl ions and
increasing the diffusion of calcium hydroxide into the
dentinal tubules.[13] Moreover, PG functions as a thickening
agent due to its ability to form intermolecular bonds, and it
can increase bond strength and reduce the setting time.[14]
These factors play an important role in the entire process
of dissociation and diffusion of calcium and hydroxyl ions
released by calcium hydroxide into dentin.[17]

In this study, the dentin elastic moduli was tested after
exposure to calcium hydroxide. The study results showed
that the modulus of elasticity of dentin exposed to the
mixture of distilled water and calcium hydroxide was 23.874
Gigapascals (Gpa), calcium hydroxide combined with
propolis was 9.529 Gpa, and the combination of the three
materials was 7.186 Gpa compared to dentin (8.242 Gpa).
These results are similar to findings of another study
stating that dentin exposed to calcium hydroxide had a
higher modulus of elasticity (21–23 Gpa) than the dentin
(15–17 Gpa).[18] The modulus of elasticity is considered as
a material stiffness measurement; the higher the modulus
of elasticity, the less change of shape when force is applied.
Thus, a greater modulus of elasticity is associated with
smaller or stiffer elastic strain. Another study concluded
that lower modulus of elasticity was associated with lower
marginal adaptation, which can lead to a gap between
restorations and teeth.[19]

Furthermore, studies show that the mechanical properties
of dentin are influenced by moisture content. Dehydrated
dentin enhances its elastic moduli, proportional limit, and
compressive strength and stress. However, dentin that
is exposed to calcium hydroxide paste has a significant
increase in its modulus of elasticity and a decrease in its
flexural strength that makes it more prone to fracture.[18,20]
This refers to the changes in the dentinal organic matrix.
Calcium hydroxide dissolves pulp tissue that originated
from denaturation and hydrolysis processes, and the high
pH of calcium hydroxide impairs the organic support of
the dentin matrix. This process weakens the interaction
between collagen threads and hydroxyapatite crystals
which adversely can affect the mechanical properties of
dentin.[21] In this study pH measurements were 12.716 for

**Table 1: The result of pH measurement**

| Treatment groups | Mean±SD | P   |
|-----------------|--------|-----|
| Ca(OH)₂ + distilled water | 12.716±0.022<sup>a</sup> | 0.000 |
| Ca(OH)₂ + propolis | 8.527±0.031<sup>b</sup> |     |
| Ca(OH)₂ + propolis + propylene glycol 40% | 7.320±0.017<sup>c</sup> |     |

Different letter showed significant difference (HSD Tukey). SD: Standard deviation, HSD: Honestly significant difference

**Table 2: The value of dentin elastic moduli**

| Treatment groups | Mean±SD | P   |
|-----------------|--------|-----|
| Ca(OH)₂ + distilled water | 23.874±0.003<sup>a</sup> | 0.000 |
| Ca(OH)₂ + propolis | 9.529±0.010<sup>b</sup> |     |
| Ca(OH)₂ + propolis + propylene glycol 40% | 7.186±0.004<sup>c</sup> |     |
| Dentin (control) | 8.242±0.005<sup>d</sup> |     |

Different letter showed significant difference (HSD Tukey). HSD: Honestly significant difference

**Table 3: Correlation between pH and dentin elastic moduli**

| Treatment groups | Dentin elastic moduli |
|-----------------|-----------------------|
|                  | Ca(OH)₂ + distilled water | Ca(OH)₂ + propolis | Ca(OH)₂ + propolis + propylene glycol 40% |
| Ca(OH)₂ + distilled water | 0.997* |           |           |
| Ca(OH)₂ + propolis |          | 0.995* |           |
| Ca(OH)₂ + propolis + propylene glycol 40% |          |          | 1.000* |
the combination of calcium hydroxide + distilled water, 8.527 for the combination of calcium hydroxide + propolis, and 7.325 for calcium hydroxide combined with propolis and PG. Similarly, other studies showed that the increase in hydrogen bonds between collagen threads can affect the stiffness and strength of teeth. This refers to the low pH that has the potential to reduce the material physical properties.

However, higher and lower pH can affect the mechanical strength of the material. Higher pH can result in higher surface strength and lower porosity of material while lower pH can change the microstructure of the material by increasing its porosity which ultimately will reduce the mechanical strength. This decrease can be referred to distribution of (OH-) ions, which can play a role in the initial setting due to the acidic atmosphere. Furthermore, the correlation between pH and the modulus of elasticity was also proven by another study showing that the more alkaline the environment, the exposed dentin will have higher elastic moduli, which means the dentin will be stiffer and more vulnerable.

**CONCLUSION**

The elastic moduli and pH value of the dentin exposed to a mixture of PG and propolis added to calcium hydroxide pH value and is smaller than the mixture of distilled water with calcium hydroxide and the mixture of propolis with calcium hydroxide. The dentin elastic moduli is correlated to its pH value; the more alkaline the environment, the exposed dentin will have higher elastic moduli.

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**Conflicts of interest**

There are no conflicts of interest.

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