ORIGINAL ARTICLE

Application of Combination Propolis Extract and Calcium Hydroxide as a Direct Pulp Capping Agent on MMP-1 Expression and Collagen Type 1 Density in Rat’s Pulp Tissue

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ABSTRACT

Introduction: Ca (OH)₂ has long been regarded as the “gold standard” of direct pulp-capping materials, Ca(OH)₂ is very soluable in oral fluid, that make tunnel defect and unable to withstand bacterial recolonization. Propolis is a material created from resin gathered by bees (Apis mellifera) from a range of plants and combined with saliva and enzymes to form a nest. Propolis possesses anti-inflammatory properties that are superior to Ca (OH)₂. Objective: The purpose of the research was carried out to integrate calcium hydroxide and propolis as pulp capping ingredients. Methods: This study used 30 samples maxillary first molars of Rattus norvegicus divided into 3 groups which were all perforated until perforation; The combination Ca (OH)₂ and propolis extract group, calcium hydroxide group, and control group. The cavity was closed with Cention. Teeth section samples were taken from rats after 3 and 7 days then underwent decalcification and histological evaluation under light microscope to identify the presence of odontoblast-like cells, inflammatory cells, and dentinal bridges. Expression of MMP-1 and Collagen type 1 density evaluated with immunohistochemistry (IHC) method. Results: According to the observations, the majority of cells in the Calcium Hydroxide and Propolis extract group showed Collagen type 1 density, whereas the least number of cells showed MMP-1. Conclusion: Compared to calcium hydroxide to a combination of Ca (OH)₂ and Propolis extract, MMP1 expression was lower and collagen type 1 density was higher in the rat’s pulp.

Keywords: Propolis extract, Calcium hydroxide (Ca(OH)₂), Direct pulp capping, MMP1, Medicine

INTRODUCTION

Dental caries is a global infectious illness that affects 72% of Indonesians, with 46.5 percent of them suffering from neglected active caries (1). This can lead to a big cavity in tooth and if caries is not treated, it can cause more infection and lead to pain. Dental pain originated from pulpa inflammation and stimulation of dental pulp nerve fibers (2). Pulpa inflammation is a pulp disease characterized by pain in the trigeminal nociceptor (3).

One of pulp inflammation is Reversible Pulpitis. Reversible pulpitis is a dental pulp inflammation that should be resolved and the pulp return to normal following appropriate management of the etiology (2). Treatment for reversible pulpitis can be directly restored but if the cavity is near or penetrating pulp indirect or direct pulp capping can be choosen (4).

Calcium hydroxide is the most commonly used material for pulp capping, although it has certain drawbacks, such as a high pH (pH 12.5), which allows the development of a tunnel defect in the dentin barrier. Calcium hydroxide is also very soluble in oral fluid (5). Based on these disadvantages, recently other materials have been submitted as a candidate for use in direct pulp capping.

Propolis is honey comb derivatives that contains flavonoid (6). Flavonoid components such as kaempferol, gallic acid, ellagic acid, catechin, quercetin, vanillic acid and others (7). Flavonoid has many functions such as anti-inflammatory, antioxidant, anti-bacterial, anti-fungal, anti-viral and anti-cancer properties, as well as the ability to accelerate wound healing (8). Caffeic Acid Phenethyl Ester (CAPE), an active ingredient found in over 50% of propolis, has biological and pharmacological properties as an anti-inflammatory and immunomodulator. CAPE has been shown to have anti-inflammatory features by inhibiting certain enzyme activities as a potent inhibitor of nuclear factor-kappa (NF-) activation and reducing COX-2 expression, a gene
derived from NF- regulation. Inhibition of NF- represents a significant role in anti-inflammatory agents because inflammation and immune systems play a critical role in the initiation of many inflammatory diseases (9). Propolis is capable of preventing inflammatory reactions caused by microorganisms and pulp necrosis, as well as stimulating the creation of dentin bridges (10). A research by Parolia (11) indicated that propolis, calcium hydroxide, and Mineral Trioxide Aggregate has similar effectiveness in inducing dentin reparative formation. Propolis-based pulp capping is more effective than calcium hydroxide-based products (12).

The advantages of propolis are the reason why the author wants to integrate natural medicine and modern medicine by using calcium hydroxide and propolis as pulp capping ingredients in the hopes that the benefits of each component can outweigh the weaknesses of other materials. After direct pulp capping procedure, odontoblast-like cells differentiated from the subodontoblast cells, creating a reparative dentin bridge in the exposed pulp tissue (11). This can be seen through MMP1 and Collagen type 1 as specific biochemical odontoblast-like functional cells (13). Based on this, it is necessary to find out MMP-1 expression and collagen type 1 density on pulp odontoblast cells after application combination of propolis extract and calcium hydroxide.

Calcium hydroxide has a pH of alkaline. When alkaline into contact with pulp tissue, it induces necrosis with a depth of 1 mm or more in the surface area of the cell, resulting in a drop in cell proliferation followed by a decrease in cell differentiation. The acidic pH of propolis extract contains active components, one of which is CAPE, a potent anti-inflammatory. The physical linkages between calcium hydroxide (alkaline) and propolis (acid), result in a pH of 7. It can prevent inflammation by inhibiting the production of proinflammatory cytokine genes (22).

MATERIALS AND METHODS

This study used animals of thirty fully grown first molar teeth of 8-16 weeks old Wistar rats, body weight 200-300 grams, feed standard (type Hi-pro-vite 524-20gram/day/head) and drinking water ad libitum. Wistar rats that have match criteria, to be samples were divided into 3 groups observations on 3rd day and 3 groups observations on day 7th day, with 5 Wistar rat in each group. Ethical approval for this study was obtained from Health Research Ethical Clearance Commission Universitas Airlangga Faculty of Dental Medicine with number 275/HRECC.FDOM/X/2018.

Combination of calcium hydroxide and propolis extract is made in dosage from hidroxido calico P.A powder and natural ingredients propolis from the Apis mellifera bee in Malang which extracted using ethanol solvent with maceration method. Preparation tools are disinfected with 95% alcohol. Ketamine (Ketalar®, Warner Lambert, Ireland) was used to anesthetize the animals. On the container, the rats are placed. Cotton pellets were used to clean the tooth’s occlusal surface, cotton pellet dipped in 95% alcohol. The maxillary right first molar was prepared in class I (classified Black) using a handpiece (: 0.46 mm) at low speed until the pulp was reached. The cavity is then dripped with a sterile saline solution and dried with a cotton pellet after perforation. Treatment group divided into 3 group. Group 1: the control group, where the teeth were prepared until the perforation, and then filled with Cention (Ivoclar Vivadent, Schaan, Liechtenstein). Group 2: the combination calcium hydroxide and aquadest (1:1), where the teeth were applied with calcium hydroxide and aquadest to the point of perforation, and then filled with Cention. Group 3: the combination calcium hydroxide and propolis extract (1:1,5), where the teeth were applied with calcium hydroxide and propolis extract to the point of perforation then filled with Cention. Rats given eat a standard meal and drink water. Rats from each treatment group will be killed with peritoneal injection in 3rd day or 7th day depend on rats groups. After taking the decapitation, the jawbone in the interdental area of the first molar the upper jaw removed.

Fixation, dehydration, and penetration, purification, paraffin infiltration, embedding, sectioning, and sticking to the object glass are all steps in the process of preparing histopathological preparations. Then, at 400x magnification, observed with a microscope light. After that, MMP1 expression and Collagen type 1 density were determined using immunohistochemistry. The average and standard deviation of the study’s findings were determined. The Kolmogorov Smirnov test was used to determine the normality of the data distribution and then homogeneity was tested. The one-way ANOVA test was used in this analysis, with a degree of significance of = 0.05, and the Tukey HSD test was used to determine differences. Figure 1 shows the overall research flow chart.

RESULTS

Histopathological examination of hematocycleine-eosin stain on rat dental pulp cells odontoblast showed the morphology of the odontoblast cells located periphery of the pulp chamber (Figure 2 and 3).

The results of immunohistochemical examination of rat pulp odontoblast cells expressing MMP1 and collagen type 1 density were seen with brown DAB staining (Figure 4 and 5).

Following immunohistochemical staining, statistical analysis was performed, with the mean and standard deviation for each sample group measured. The Kolmogorov Smirnov normality test revealed a value of
Figure 1: Research Flow Chart

Figure 2: Inferior ranium and maxilla of rats (A), maxillary molar from rats is seen with a microscope 40x magnification (B)

Figure 3: Morphology of rat dental pulp odontoblast cells. Black arrows indicate histopathological features odontoblast morphology in rat dental pulp with 100x (a) and 400x (b) magnification, black spot is the odontoblast in the dentin of pulp chamber.

Figure 4: Pulp odontoblast cells of a rat express MMP-1 (400x). Black arrow mark showed the expression of MMP1 on the 3rd day (A) MMP1 expression on the 3rd day after it applied by the Cention (control). Brown stain indicated expression of MMP1, it has more colored in this picture. (B) Expression of MMP1 the 3rd day after it applied by calcium hydroxide. Brown stain indicated expression of MMP1, the brown stain less than picture a. (C) MMP1 expression on the 3rd day after it had applied combination calcium hydroxide and propolis extract. In this picture the brown stain is the least visible.

Figure 5: Pulp odontoblast cells of a rat express MMP-1 (400x). Black arrow mark showed the expression of MMP1 on the 7th day. Group 1, Group 2, and group 3. (A) MMP1 expression on the 7th day after it applied the Cention (control). Brown stain indicated expression of MMP1, it has more colored and longer in this picture. (B) Expression of MMP1 7th day after it applied by calcium hydroxide. Brown stain indicated expression of MMP1, the brown stain less than picture a. (C) MMP1 expression on the 7th day after it had applied combination calcium hydroxide and propolis extract. In this picture the brown stain is smaller than others.
From the data analysis expression of MMP1 on day 3 and day 7, the group of combination calcium hydroxide and propolis extract showed a lower expression of MMP-1 compared to group 1 and group 2. This means that there is a significant decrease in inflammation in the combination calcium hydroxide and propolis extract group compared to group 1 and group 2 (Figure 6 and 7).

DISCUSSION
Calcium hydroxide is the most common pulp capping substance used in dentistry. Calcium hydroxide was chosen as a pulp capping material because of its ability to promote event mineralization. However, calcium hydroxide has drawbacks, including the potential to irritate and inflame the pulp. The inflammatory response is the first step in a sequence of healing processes. When tissue is injured, fibroblast cells are stimulated to migrate to wounds, proliferate, and create a large amount of collagen matrix, which aids in the isolation and repair of damaged tissue (14).

MMP1 plays a role in matrix degradation during injury for tertiary dentin formation, and plays a role in pulp inflammation. MMP-1 can be induced by inflammation, under normal physiological conditions MMP-1 is expressed at low levels, but increased expression as an indicator of pathological conditions. (15)

Odontoblast cells are one of the many types of cells found...
in pulp tissue. It is said to be pulp tissue-specific since it is crucial to the function of the dentin and pulp. Forms a coating that will eventually be mineralized into dentin. Odontoblasts perform a formative as well as a reparative role for the dental pulp during its existence, namely the formation of primary, secondary, and tertiary dentin (16). The odontoblast cells would be destroyed if perforation occurs. The odontoblast cells in the pulp are mature cells that have reached the end of their differentiation process and are unable to replace damaged cells. The process of differentiation of fibroblast cells contained in the pulp tissue will produce new odontoblast cells. Form 1 collagen will be produced by these odontoblast cells. Collagen type 1 plays a critical role in the formation and mineralization of reparative dentin (14).

In this study, the propolis extract was combined with calcium hydroxide as a pulp capping material because of its anti-inflammatory ability, so that the pulp repair process characterized by the formation of dentin bridges can occur better. Calcium hydroxide and propolis extract were combined with a ratio of 1:1.5 based on preliminary research on viability and setting time.

On day 3, there was a significant difference between the mean value of MMP1 and collagen type 1 expression in the Cention control group compared to the calcium hydroxide group with propolis extract, but no significant difference was found between the Cention control group and the calcium hydroxide group. This shows that on day 3, the calcium hydroxide group has not shown good repair because there is still inflammation in the necrotic area of the pulp tissue that is in direct contact with calcium hydroxide. This assumption is supported by the theory that on day 3 histologically there is inflammation with a large number of neutrophils, lymphocytes, and macrophages, and moderate to severe degrees of inflammation in pulp cells (17).

The expression of MMP1 on the 3rd and 7th day of the calcium hydroxide group with propolis extract showed the lowest mean value with significant differences compared to the calcium hydroxide group with distilled water and the Cention control group. The lower mean of MMP1 expression in the calcium hydroxide group with propolis extract was due to the fact that the active ingredient content of propolis, namely CAPE (caffeic acid phenethyl ester), can cause a significant reduction in the production of proinflammatory cytokines such as TNF-α and MMP-1 which will be expressed in response to this damage. But in contrast, it increases the production of TGF-β which is an anti-inflammatory cytokine through its effect on the activation of the transcription factor NF-kβ from the MAP kinase transduction pathway (18). This proinflammatory and pro wound healing environment causes pulp repair to occur better in the calcium hydroxide group with propolis extract.

On day 7, the expression of collagen type 1 in the calcium hydroxide group was less than the calcium hydroxide-propolis combination group with significant differences. Calcium hydroxide has anti-bacterial properties because it has a high pH of 12.5 so it can destroy cell membranes and protein structures.

Calcium hydroxide consists of Ca2+ and OH ions. Hydroxyl ion enters the cell via calcium channel which induces phosphorylation of Kappa Inhibitor β (Ikβ), then Ikβ degrades rapidly and liberates NF-kβ and is followed by an increase in tumor necrosis factor α (TNFα) and MMP-1 (19). When calcium hydroxide comes into contact with pulp tissue, it induces necrosis with a depth of 1 mm or more in the surface area of the cell, resulting in a drop in cell proliferation followed by a decrease in cell differentiation, resulting in a drop in type 1 collagen synthesis (20).

Propolis has been studied in vitro and in vivo, and the results suggest that it has a variety of biological and pharmacological properties, as well as being an anti-inflammatory agent (21). The inclusion of caffeic acid and phenethyl ester (CAPE) in propolis gives it anti-inflammatory properties. Propolis ethanol extract has good endodontic qualities, such as boosting bone regeneration and boosting the creation of hard bridge tissue in pulpotomy or pulp capping procedures (22). Flavonoid chemicals, amino acids, terpenes, and cinnamic acid derivatives all contribute to propolis' anti-inflammatory capabilities. The fact that propolis suppresses eicosanoid synthesis is the method by which it reduces inflammation. The level of arachidonic acid in the cell membrane phospholipid will drop as a result of this inhibition, which will further inhibit the production of inflammatory mediators including prostaglandins, leukotrin, and thromboxane (21).

The calcium hydroxide-propolis combination group's collagen type 1 expression enters the cytoplasmic membrane via passive transport (diffusion). Calcium hydroxide is made up of calcium ions and hydroxyl ions, and it has a pH of alkaline. The acidic pH of propolis extract contains active components, one of which is CAPE, a potent anti-inflammatory. The physical linkages between calcium hydroxide (alkaline) and propolis (acid), especially hydrogen and Van Der Waals bonds, result in a pH of 7. Calcium hydroxide and propolis extract have anti-inflammatory activities via reducing NF-k activation, which inhibits the signal that promotes phosphorylation Ik. If NF-k is not released, it can prevent inflammation by inhibiting the production of proinflammatory cytokine genes (23).

When calcium hydroxide and propolis ph 7 extract were mixed, TGF- levels improved. TGF- aids pulp cells proliferate and grow into young fibroblasts by increasing their proliferative activity. Young fibroblasts can develop into odontoblast-like cells and increase collagen type 1 expression to replace damaged odontoblasts (24).
Based on observations of MMP1 and collagen type 1 expression, it can be determined that the calcium hydroxide group with propolis provided the best repair effect when compared to the calcium hydroxide group and the Cention control group.

CONCLUSION

The expression of MMP1 in the combination application of calcium hydroxide with propolis extract on the 3rd and 7th day was lower than that of calcium hydroxide. The density of type 1 collagen in the combination application of calcium hydroxide with propolis extract on the 3rd and 7th day was higher than that of calcium hydroxide.

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REFERENCES

1. Pribadi N, Effendy R, and Ruslianda D A. The Difference Between the Amount of Glucose as the Product of Metabolism of Glucosyltransferase Enzyme Streptococcus Mutans In Neutral pH and Optimal pH. International Medical Device and Technology Conference. 2017: 121-123