

Original article:

The Comparative Study Between Calcium Hydroxide and Garlic Extract on Inhibitory Effect of Clinical Isolate Bacterial of Primary Teeth

IAIA Krisna Kencana Dewi¹, I Bramanti², ISR Sudarso³, MSH Wahyuningsih⁴, T Wibawa⁵

Abstract:

This study aimed to compare the antibacterial effectivity of calcium hydroxide and garlic extract by assessed the inhibitory effect on clinical isolate bacterial of necrotic primary teeth. **Materials and methods:** Clinical isolate bacterial of primary teeth were taken from the children who visited the Clinic of Pediatric Dentistry, Dental Hospital of UGM Prof Soedomo, Yogyakarta, Indonesia with age 4-7 years old. Aerobic and anaerobic bacteria were taken directly from 26 children who will undergo endodontic treatment with a diagnosis of pulp necrosis. The identification and isolation technique was done based on *CLSI Standardized Method 2012* and *Biochemical identification by Thermo Scientific RapID Systems 2010*. There are 3 clinical isolates bacterial of necrotic primary teeth as reference for *in vitro* antibacterial activity testing and 2 bacteria as a comparison. Antibacterial effectivity of calcium hydroxide was examined by diffusion method by comparing calcium hydroxide, 20%, 40%, 60%, 80% concentration of garlic extract, and garlic filtrate. The sensitivity test result was taken after 24-48 hours of incubation by assessing the inhibitory zones around the well. **Results:** The measurement result of inhibitory diameter zone of Calcium hydroxide against *Prevotella bivia* was 30 mm, against *Clostridium innocum* was 30 mm, against *Serratia marcescens* was 23 mm. The inhibitory diameter zone of Calcium hydroxide against *Clostridium perfringens* as a comparative bacteria was 34 mm and 30 mm against *Streptococcus mutans*. **Conclusion:** All groups in this study have inhibitory effect against clinical isolate bacterial of necrotic primary teeth. The inhibitory effect of calcium hydroxide is greater than the inhibitory effect of garlic extract but lower when compared with garlic filtrate.

Keywords: Calcium hydroxide, garlic, clinical isolate bacterial of necrotic primary teeth.

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Introduction

Dental caries is one of the most prevalent problems which always occur in the oral cavity especially in children. Based on the Global Burden of Disease Study 2016 estimated that 2.4 billion people suffer from caries of permanent teeth and 486 million children suffer from caries of primary teeth¹. Basic Health Research of Indonesia 2018 stated that 93% of early childhood in Indonesia with the age of 5-6 years have dental caries². Untreated dental caries will spread and affect dental pulp tissue which can affect the vitality of the tooth and it lead to pulp

necrosis³. Pulp necrosis is a clinical diagnostic category indicating non-vitalisation of the dental pulp caused by untreated pulp inflammation. One contributing factor that leads to pulp necrosis is microbes. Zehnder (2006) stated that root canal infection is polymicrobial and involved anaerobic gram-positive and gram-negative bacteria. Obligate and facultative anaerobe bacteria, microaerophilic bacteria and fungi also found in pulp necrosis⁴.

Untreated pulp necrosis will disrupt the mastication process thus it can affect the intake

1. IAIA Krisna kencana Dewi, Student of Post Graduate Programme, Faculty of Dentistry UGM, Indonesia
2. I Bramanti, Lecturer of Pediatric Dentistry Department, Faculty of Dentistry, UGM, Indonesia
3. ISR Sudarso, Lecturer of Pediatric Dentistry Department, Faculty of Dentistry, UGM, Indonesia
4. MSH Wahyuningsih, Lecturer of Pharmacology and Therapy Departement, Faculty of Medicine, UGM, Indonesia
5. T Wibawa, Lecturer of Microbiology Departement, UGM, Indonesia

Correspondence to: Bramanti I, Lecturer of Pediatric Dentistry Department, Faculty of Dentistry UGM Yogyakarta, Indonesia. Jl. Denta 1, Sekip Utara, Yogyakarta, 55281, Indonesia, email: bramantikg@ugm.ac.id

of nutrients which is important for the growth and development of the children⁵. Endodontic treatment or root canal treatment is one of the treatment indicated for pulp necrosis. The main purpose of this treatment is to eliminate the causative microorganisms and prevent recurrent infections⁶. Biomechanical preparation does not ensure the complete elimination of microorganisms present in the root canal system⁷. Root canal sterilization is one of the stages in root canal treatment carried out by root canal irrigation and the administration of root canal medicament. The addition of root canal medicament aims to eliminate the microorganisms that remain during the chemomechanical process⁸.

Calcium hydroxide has been widely used in endodontic treatment as an intracanal medicament and its history of use over a long period in the endodontic field has established its safety⁹. Calcium hydroxide routinely uses especially for root canal treatment in primary teeth because of its high pH (approximately 12.5) thus has a great antibacterial activity⁷. Antimicrobial properties of calcium hydroxide are attributed to the release of hydroxyl ions that make highly alkaline environment. Most of the microorganisms from an infected root canal are unable to survive in the alkaline environment¹⁰. However, calcium hydroxide unable to eliminate all bacteria found in the root canal system even though it has great antibacterial activity. Some study reported about calcium hydroxide failure to eliminate some root canal bacteria that caused reinfection of root canal system¹¹. This study aimed to reevaluate the antibacterial effectivity of calcium hydroxide by assessed the inhibitory effect on clinical isolate bacterial of primary necrotic teeth. Calcium hydroxide commonly used in endodontic treatment as a drug of choice for root canal medicament in the tooth with pulp necrosis thus the antibacterial effectivity evaluation of calcium hydroxide was done by compared calcium hydroxide with herbal medicine. Garlic is one of the herbal medicine which potentially as an intracanal medicament. Antibacterial effectivity of calcium hydroxide was evaluated by comparing it with garlic extract.

Materials and methods

In this study clinical isolate bacterial of primary teeth were taken from the children who visited the Clinic of Pediatric Dentistry, Dental Hospital of UGM Prof Soedomo, Yogyakarta, Indonesia with age 4-7 years old. Aerobic and anaerobic bacteria were taken directly from 26 children

who will undergo endodontic treatment with a diagnosis of pulp necrosis. The study protocol was accepted and approved by the Ethics and Advocacy Commission of the Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia. The identification and isolation technique was done based on *CLSI Standardized Method 2012* and *Biochemical identification by Thermo Scientific RapID Systems 2010*. There are 3 clinical isolates bacterial of necrotic primary teeth as reference for *in vitro* antibacterial activity, namely *Prevotella bivia*, *Clostridium innocum*, and *Serratia marcescens*. There are 2 bacterias as comparison bacteria, namely *Streptococcus mutans* ATCC35688 as a comparison of *Serratia marcescens* and *Clostridium perfringens* obtained from the Laboratory Office of Yogyakarta Regional as a comparison of *Clostridium innocum* dan *Prevotella bivia*.

The antibacterial effectivity of calcium hydroxide was evaluated with diffusion method by compared calcium hydroxide, garlic extract, and garlic filtrate. The Calcium hydroxide used in this study is root canal dressing material obtained by mixing calcium hydroxide powder with a glycerin solution in ratio 1:1 to obtain paste consistency. The garlic in this study is garlic from "Tawangmangu Baru" variety. Garlic extract was obtained by maceration method with ethanol 96% solvent. The garlic extract effectivity was evaluated in 20%, 40%, 60% and 80% of concentration. Garlic filtrate was obtained by mashing garlic then filtered with sterile gauze. The sensitivity test result for aerobic and anaerobic bacterias was assessed after 24-48 hours of incubation in 37⁰ C of temperature. The antibacterial effectivity of calcium hydroxide was evaluated by assessing the inhibitory zones of clinical isolate primary necrotic teeth bacterias. The measurements were made by examined the inhibitory zones which are the clear area around the well containing the experimental solution. The diameter of clear zones was measured by zonameter from the center of the well to the edge of inhibitory zones, then the three materials were compared.

Results

The comparison result of the inhibitory zone diameter of calcium hydroxide, garlic extract with 20%, 40%, 60%, 80% concentration and garlic filtrate against *Serratia marcescens* as the clinical isolate bacteria of primary necrotic teeth (Table 1.) and against *Streptococcus mutans* ATCC35688 as the comparison bacteria (Table 2.).

Table 1. The diameter of inhibitory zone againsts *Serratia marcescens*

No	Materials	Diameter of Inhibitory Zone (mm)
1	GE 20%	0
2	GE 40%	0
3	GE 60%	0
4	GE 80%	10
5	Garlic juice	31,5
6	Calcium hydroxide	23

*GE = Garlic extract

Table 2. The diameter of inhibitory zone againsts *Streptococcus mutans* ATCC35688

No	Materials	Diameter of Inhibitory Zone (mm)
1	GE 20%	0
2	GE 40%	0
3	GE 60%	0
4	GE 80%	0
5	Garlic juice	49
6	Calcium hydroxide	30

*GE = Garlic extract

Base on table 1. result of the inhibitory effect of calcium hydroxide against *Serratia marcescens* was 23 mm. It showed a higher number compare to garlic extract which the diameter of the inhibitory zone was 10 mm in 80% concentration, however if the calcium hydroxide was compared with garlic filtrate the inhibitory zone of calcium hydroxide was lower. Table 2. Showed the measurement result of inhibitory zone of calcium hydroxide against *Streptococcus mutans* ATCC35688 which has 30 mm of diameter. This result showed a higher number if compared with garlic extract against *Streptococcus mutans* ATCC35688.

The comparison result of inhibitory zone from calcium hydroxide, 20%, 40%, 60%, 80% concentration of garlic extract and garlic filtrate against *Prevotella bivia* the bacteria of clinical isolate primary necrotic teeth presented in table 3. against *Clostridium innocum* presented in table 4. And against *Clostridium perfringens* as a comparison bacteria presented in table 5.

Table 3. The diameter of inhibitory zone againsts *Prevotella bivia*

No	Materials	Diameter of Inhibitory Zone (mm)
1	GE 20%	0
2	GE 40%	0
3	GE 60%	0
4	GE 80%	0
5	Garlic juice	49
6	Calcium hydroxide	30

*GE = Garlic extract

Table 4. The diameter of inhibitory zone againsts *Clostridium innocum*

No	Materials	Diameter of Inhibitory Zone (mm)
1	GE 20%	0
2	GE 40%	0
3	GE 60%	0
4	GE 80%	0
5	Garlic juice	28
6	Calcium hydroxide	0

*GE = Garlic extract

Table 5. The diameter of inhibitory zone againsts *Clostridium perfringens*

No	Materials	Diameter of Inhibitory Zone (mm)
1	GE 20%	0
2	GE 40%	0
3	GE 60%	0
4	GE 80%	0
5	Garlic juice	46
6	Calcium hydroxide	34

*GE = Garlic extract

The measurement result of inhibitory zone diameter of calcium hydroxide against *Prevotella bivia* was 30 mm, against *Clostridium innocum* was 0 mm and against *Clostridium perfringens* was 34 mm. This result showed the higher number if compared with garlic extract except for *Clostridium innocum* group, it showed the same diameter. However, the calcium hydroxide diameter of the inhibitory zone still lowers when compared with garlic filtrate.

Discussion

The bacterial identification of root canal in necrotic primary teeth and evaluation of the antibacterial effectivity of calcium hydroxide compared with garlic extract was done in this study. Further, 3 types of necrotic primary teeth isolate bacteria was determined as a reference for the examination of antibacterial activity in vitro, namely *Prevotella bivia*, *Clostridium innocum* and *Serratia marcescens*. *Prevotella bivia* is a type of anaerobic gram-negative rods bacteria. This goes in hand with research done by Praveen et al. (2018) confirming the relationship of *Prevotella spp.* with the occurrence of deep dentinal caries in the age group mixed dentition¹². This is also supported by research on the prevalence of pathogenic bacteria from dental caries which has found 12.64 % of *Prevotella* species out of total sample¹³. On the other side, *Clostridium innocuum* which is a type of gram-positive anaerobic bacteria was also found¹⁴. There are several studies have proven the existence of *Clostridium* species that can cause dentoalveolar abscesses in various variations from 2% to 20%¹⁵. *Serratia marcescens* is also one of the bacteria found in necrotic deciduous tooth isolates in this study.

In addition, it is seen from the results of the antibacterial effectivity of calcium hydroxide, garlic extract and garlic filtrate have shown that all groups had an inhibitory effect against clinical isolate bacteria of necrotic primary teeth and ATCC bacteria as a comparison. The results showed that calcium hydroxide has a greater inhibitory effect compared with garlic extract. Kim (2014) on a study of the antimicrobial effects of calcium hydroxide stated that calcium hydroxide is effective in eliminating the bacteria, this possibly happens as calcium hydroxide has a high pH (around 12.5), causing destructive effects on cell membranes and cell structure^{11,16}. The process of alkalization can occur due to the antimicrobial action of calcium hydroxide associated with the release of hydroxyl ions. These ions work as high reactive free radicals that cause lethal effects on bacteria⁷. Other studies suggest the lethal effect of hydroxyl ions on bacterial cells could be due to mechanisms such as damage to the cytoplasmic membrane from bacteria, denaturation of proteins and damage to bacterial DNA. However, it is not yet known exactly which effect plays the most role among the three¹⁷.

It is seen that calcium hydroxide shows a better effect when compared to garlic extract in concentrations of 20%, 40%, 60%, 80% but when compared to garlic filtrate, calcium hydroxide shows lower results. One of the active substances contained in garlic as an antimicrobial in addition to essential oils is *Alisin*. It is believed as effective to eliminate the gram-positive and negative bacteria¹⁸. On the contrary, in a research conducted by Eswar et al. (2013) regarding dentinal tubule disinfection with chlorhexidine, garlic extract, and calcium hydroxide found that garlic extract has a better effect when compared to calcium hydroxide in fighting against *Enterococcus faecalis*. Garlic extract was obtained by mixing 100 grams of garlic with 125 mL distilled water which was filtered then juiced and crushed before it was centrifuged at a speed of 10,000 rpm for 20 minutes to obtain a concentration of 249 mg / mL¹⁹. In the process of obtaining garlic extract, there may be some components that are missing or reduced so that the garlic in the form of juice shows better results. It can be concluded that further research needs to be conducted about the ingredients that play the most role in garlic as an antibacterial agent.

Conclusion

All groups in this study have inhibitory effect against clinical isolate bacterial of primary necrotic teeth. The inhibitory effect of Calcium hydroxide is greater than the inhibitory effect of garlic extract but lower when compared with garlic filtrate.

Acknowledgement

Ethical clearance

The study protocol was accepted and approved by the Ethics and Advocacy Commission of the Faculty of Dentistry, Gadjah Mada University, Yogyakarta, Indonesia.

Conflict of Interest

The authors deny any conflicts of interest related to this study.

Author's contribution

All authors have contribution to this study. First and Second author has involved in making idea, gathering (collect and analyzed) data and also involved in whole stages of publication process. Third, 4th and 5th author contributed for editing and approval of final draft.

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