Research Report

Antimicrobial activity of calcium hydroxide, calcium oxide, and mineral trioxide aggregate paste against α-Hemolytic *Streptococcus*

Eveline Yulia Darmadi[1], Diana Soesilo[2]
[1]Division of Conservative Dentistry, Faculty of Dental Medicine, Universitas Ciputra, Surabaya, Indonesia
[2]Department of Conservative Dentistry, Faculty of Dentistry, Universitas Hang Tuah, Surabaya, Indonesia

ABSTRACT

Background: *The bacteria mostly found in the root canals are aerobic and facultative anaerobic bacteria, such as α-Hemolytic Streptococcus. Calcium hydroxide has alkaline characteristics with pH of 12.5, and antimicrobial properties. Calcium oxide is hydrophilic and has a smaller molecular weight than calcium hydroxide. Mineral trioxide aggregate (MTA) has alkaline properties, good biocompatibility, stimulates healing and inhibits bacterial growth.* **Purpose:** *The purpose of this study was to examine the differences of antimicrobial activity between calcium hydroxide, calcium oxide, and mineral trioxide aggregate (MTA), also to find which of these materials has the highest antimicrobial activity.* **Methods:** *The design of this study was a post-test only control group design. The study subjects were divided into 4 groups. K (-) was control group without any treatment, P1 was treated with calcium hydroxide, P2 was treated with calcium oxide, and P3 was treated with MTA. Each group consisted of 7 samples. The α-Hemolytic Streptococcus were spread on Mueller Hinton agar. Antimicrobial test was using diffusion method and diameter of inhibition zones were measured with caliper.* **Results:** *Average inhibitory zone of each sample was P1 (30.9643 mm ± 0.7431), P2 (35.2357 mm ± 0.7099), P3 (28.6 mm ± 1.5532). Tukey HSD test showed significances results between samples P1 and P2 (p=0.001), P1 and P3 (p=0.002), P2 and P3 (p=0.001).* **Conclusion:** *The highest antimicrobial activity against α-Hemolytic Streptococcus was calcium oxide followed by calcium hydroxide and MTA.*

Keywords: *antimicrobial activity, calcium hydroxide, calcium oxide, mineral trioxide aggregate, α-Hemolytic Streptococcus*

Correspondence: Eveline Yulia Darmadi, Division of Conservative Dentistry, Faculty of Dental Medicine, Universitas Ciputra, Citraland CBD Boulevard, Sambikerep, Surabaya 60219, Indonesia. E-mail: eveline.darmadi@ciputra.ac.id

INTRODUCTION

Previous researches have been done to seek materials with potent antibacterial activity.[1-3] These materials may come from marine resources.[4,5] The materials may also come from other natural resources.[6-8] The bacteria most often found in root canals are anaerobic, aerobic and facultative anaerobic bacteria, such as α-Hemolytic *Streptococcus*. The α-Hemolytic *Streptococcus* is one of bacteria in the root canals which is gram positive and has the form of a coccus with long chains.[9] Root canal bacteria adhere to the dentin walls or penetrate the dentin tubules. Bacteria attached to dentin are easier to kill than in dentin tubules, but bacteria in dentin tubules can also be killed with a paste that has antimicrobial properties.[10]

One of the ingredients often used in root canal treatment is calcium hydroxide. Calcium hydroxide paste was introduced in the field of dentistry in 1920 as a treatment and filling of root canals. Calcium hydroxide is also an ingredient used for direct or indirect pulp capping treatment, prevention of root resorption, root perforation, root fracture and apexification.[11] Although calcium hydroxide may affect the viability of cells, it can regulate anti-inflammatory cytokine.[12-14] Treatment with calcium hydroxide can cure periapical infections, cease pus formation, reduce pain, has a pH of 12.5 so it is alkaline, and also has antimicrobial properties. Calcium hydroxide releases hydroxyl ions which can cause damage to the cytoplasmic membrane of bacteria, then the process of protein denaturation occurs, and inhibits DNA replication of bacteria, thereby inhibiting bacterial growth. Calcium hydroxide paste can inhibit the growth of *Streptococcus mutans, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Bacillus subtilis, Enterococcus faecalis, and Porphyromonas gingivalis*.[15-17]

Persistent infections sometimes occur and are one possibility due to the presence of germs in the dentin tissue. Bacteria and their products are also found in many dentinal tubules and accessory canals; therefore, research was developed until the discovery of the calcium oxide technique based on biological and chemical principles.[10]

Calcium oxide will expand in the tooth to fill the root canals and dentinal tubules so that it can reach areas that are difficult for other materials to reach. Calcium oxide paste has been used in Europe for 15 years for the treatment of pulp infections. The use of calcium oxide is better than calcium hydroxide, this is because calcium oxide is hydrophilic and has a smaller molecular weight than calcium hydroxide. Its antimicrobial ability is obtained from the binding reaction of H_20 in dentin tubules, which then forms calcium hydroxide. Release of hydroxyl ions occurs, then neutralizes the acidic pH of the bacteria, which can cause damage to the cytoplasmic membrane of the bacteria, then the protein denaturation process occurs, inhibiting DNA replication of the bacteria, lysing necrotic tissue and releasing CO_2, thereby inhibiting bacterial growth. In addition, calcium oxide is effective in shortening the healing time of periapical lesions before root canal filling and is also effective in inhibiting bacterial growth.[11,18]

Along with technological developments in the field of dentistry, mineral trioxide aggregate (MTA) was discovered. This MTA was developed in 1993 by Torabinejad and Pitt-Ford (Loma Linda University Endodontics Section, California, United States). This material can be used for pulp capping, pulpotomy, furcation perforation repair, prevention of root resorption, root canal filling, and apexification. MTA has alkaline properties, good biocompatibility, stimulates healing and inhibits bacterial growth.[19] MTA contains calcium oxide, which when in contact with water, calcium oxide changes into calcium hydroxide. This material has a pH between 10 and 12.5 which is similar to calcium hydroxide paste, this high pH of MTA can inhibit bacterial growth by releasing hydroxyl ions.[20] This study was aimed to examine the inhibitory activity of root canal paste containing calcium hydroxide, calcium oxide, and mineral trioxide aggregate (MTA) against α-Hemolytic *Streptococcus*.

MATERIALS AND METHODS

Tools used in this research were sterilized using a dry heat sterilizer. The α-Hemolytic *Streptococcus* bacteria were transferred in BHIB (Brain Heart Infusion Broth) media with a loop. Then 0, 1 cc was taken with a micropipette, taken into 25 ml of Mueller Hinton Agar medium, then spread evenly over the Mueller Hinton Agar medium with a spreader, then onto the medium 4 wells were made in each petri dish by placing a platinum ring with a diameter of 5 mm and a 9 mm height. The ring is placed using tweezers heated with spiritus burner. The first well was the control group (without treatment), the second well was filled with calcium hydroxide paste, the third well was filled with calcium oxide, and in the fourth well was filled with MTA. Samples were incubated at 37^{o}C for 24 hours.

The results were done by observing and calculating the area of the inhibition zone. Measurement of the inhibition zone was done by measuring the diameter of the inhibition zone using a caliper which has an accuracy of 0.05 mm. Method of measurement: the diameter of the inhibition zone was measured using a caliper from two different sides and then the average was taken. Measurement of the inhibition zone was done by taking two mutually perpendicular lines through the center of the well hole, while a third line was taken between these two lines, forming an angle of 45°. Measurements were carried out three times at the same place.

The data obtained was analyzed for normal distribution using the Shapiro Wilk test and the homogeneity of the variants using the Levene test. One-Way ANOVA test was used to see the difference in the inhibition zone of root canal pastes: calcium hydroxide, calcium oxide, and MTA against α-Hemolytic *Streptococcus* and to determine the significance of the differences between calcium hydroxide, calcium oxide, and MTA root canal pastes against α-Hemolytic *Streptococcus* using the Tukey HSD test.

RESULTS

Antimicrobial inhibitory test of root canal paste containing calcium hydroxide, calcium oxide and MTA against α-Hemolytic *Streptococcus* was carried out on seven samples, each sample was divided into four groups. K (-) was the control group of α-Hemolytic *Streptococcus* without any treatment, P1 was sample group of α-Hemolytic *Streptococcus* with calcium hydroxide paste, P2 was sample group of α-Hemolytic *Streptococcus* with calcium oxide paste and P3 was sample group of α-Hemolytic *Streptococcus* with MTA paste (Figure 1).

Root canal paste containing calcium oxide reaches a higher zone of resistance compared to root canal paste containing calcium hydroxide and MTA. The calcium oxide group has the largest mean zone of inhibition against α-Hemolytic *Streptococcus* compared to calcium hydroxide and MTA

Table 1 showed that the results of the data distribution test using the Shapiro Wilk test statistical test for all groups had p value > 0.05. This showed that all groups of inhibitory

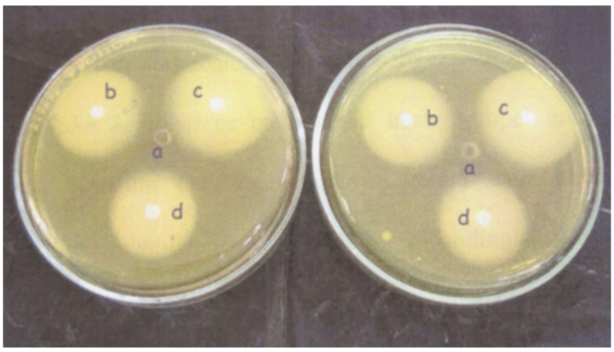

Figure 1. Inhibitory zone of α-Hemolytic *Streptococcus* on Mueller Hinton Agar medium which was divided into 4 groups: a. K (-) without any treatment, b. P1 with calcium hydroxide paste, c. P2 with calcium oxide paste, d. P3 with MTA paste.

DOI: https://doi.org/10.20473/cdj.v14i1.2024.24-28

Table 1. The p value of the results of the Shapiro Wilk test and Levene test of the inhibition zone against α-Hemolytic *Streptococcus*

Sample Group	Shapiro Wilk test	Levene test
Calcium hydroxide	p = 0.993 *	
Calcium oxide	p = 0.758 *	p = 0.139 *
MTA	p = 0.815 *	

Table 2. Tukey HSD test results for the inhibition zone against α-Hemolytic *Streptococcus*

Sample Group	Calcium hydroxide	Calcium oxide	MTA
Calcium hydroxide	-	p = 0.001 *	p = 0.002 *
Calcium oxide	-	-	p = 0.001 *
MTA	-	-	-

zone measurements against α-Hemolytic *Streptococcus* have normal data distribution. Meanwhile, the results of the homogeneity of variance test using the Levene test statistical test have a p value > 0.05. This showed that the three groups of inhibitory zone measurements for α-Hemolytic *Streptococcus* had homogeneous variances.

The One-Way ANOVA test was carried out to determine differences in inhibition zones against α-Hemolytic *Streptococcus* between groups which obtained p value = 0.001 ($p < 0.05$). This shows that there was a significant difference in the zone of inhibition against α-Hemolytic *Streptococcus* between the three groups. To determine the level of significance between the three groups, the Tukey HSD statistical test was used. The results obtained were as shown in Table 2 that the results of the difference test between all groups had a p value <0.05. This showed that there was a significant difference in the zone of inhibition against α-Hemolytic *Streptococcus* between the three groups.

DISCUSSION

Calcium oxide is hydrophilic and alkaline. The binding reaction of water with calcium oxide forms calcium hydroxide. Release of hydroxyl ions occurs, neutralizing the acidic pH of bacteria, which can cause damage to the cytoplasmic membrane of bacteria, causing lipid peroxidation and inhibiting the work of enzymes that play a role in the growth and metabolism of bacteria in the cytoplasmic membrane in tissues through the activity of alkaline phosphate. This enzyme is important for inhibiting the work of bacterial enzymes. The release of hydroxyl ions causes protein denaturation, inhibits DNA replication, lyses necrotic tissue and releases carbon dioxide, thus inhibiting bacterial growth. Calcium oxide paste contains zinc oxide, where zinc oxide also has bacterial inhibitory power by damaging cell proteins and bacterial enzymes.[11,21]

Calcium hydroxide paste has inhibitory power against α-Hemolytic *Streptococcus* bacteria due to the release of hydroxyl ions, which will come into contact with the bacteria resulting in hydrolysis of the polysaccharide lipids from the bacteria, this will increase the permeability of the bacterial cell membrane, then the process of protein denaturation, inactivation of enzymes and inhibiting DNA replication occurs, so, it will inhibit the growth of bacteria.[22] The bacterial inhibitory power of calcium hydroxide paste is directly influenced by the release of hydroxyl ions. Calcium hydroxide can inhibit bacterial growth because it is bacteriostatic. The release of hydroxyl ions inactivates bacterial lipopolysaccharide. Bacterial lipopolysaccharide possesses wide range of effects to the periapical tissue destruction.[23,24] The inactivation of bacterial lipopolysaccharide reduces the local inflammatory response, increases the solubility of necrotic tissue, and creates an alkaline environment around the tissue.[25]

Mineral trioxide aggregate paste has a similar inhibitory reaction to calcium hydroxide. The release of hydroxyl ions is obtained from calcium oxide which comes into contact with water to form calcium hydroxide which then releases calcium ions and hydroxyl ions.[26,27] With the release of hydroxyl ions, it will damage the cells cytoplasmic membrane, then a protein denaturation process occurs, thereby inhibiting bacterial DNA replication which will inhibit bacterial growth.[28]

The inhibitory power of mineral trioxide aggregate paste was smaller than calcium oxide paste and calcium hydroxide paste, because inhibiting bacterial growth was obtained from the release of hydroxyl ions resulting from calcium dissolution. The calcium content in pasta containing calcium oxide is 71.4%; in pasta containing calcium hydroxide of 54.11%; and MTA paste by 50%. This content affects pH in releasing hydroxyl ions. The greater the calcium ions that are released, the greater the hydroxyl ions that are produced.[29]

Things that can influence the way antimicrobial properties work are the speed of dissociation and diffusion of a drug, membrane permeability, buffer capacity, pH and hydroxyl ions of a drug, and viscosity. These factors also influence the size of the obstacle zone.[30] Drugs that easily experience diffusion and dissociation will produce high bacterial inhibition power and produce large inhibition zones. The mechanism of action of antimicrobial drugs is to inhibit bacterial cell synthesis, inhibit the formation of bacterial cell walls, inhibit protein wall synthesis, inhibit bacterial cell nucleic acid synthesis and disrupt bacterial permeability.[31,32] This study is preliminary where complexity of the root canal system that affect the efficacy of these materials in application is not considered. Further studies are suggested with advanced techniques or more complex root canal systems.

In conclusion, there were differences in the inhibitory activity among calcium oxide, calcium hydroxide, and MTA paste against α-Hemolytic *Streptococcus*. Calcium oxide paste had the greatest inhibitory activity compared to calcium hydroxide paste and MTA paste against α-Hemolytic *Streptococcus*, while calcium hydroxide paste has a greater inhibitory activity compared to MTA paste against α-Hemolytic *Streptococcus*.

REFERENCES

1. Astuti RHN, Samadi K, Prasetyo EP. Antibacterial activity of *Averrhoa bilimbi* Linn extract against *Enterococcus faecalis*. Conservative Dentistry Journal 2016; 6(2): 93-8.
2. Juniarti DE, Kusumaningsih T, Soetojo A, Prasetyo EP, Sunur YK. Antibacterial activity and phytochemical analysis of ethanolic purple leaf extract (*Graptophyllum Pictum* L Griff) on *Lactobacillus acidophilus*. Malaysian Journal of Medicine and Health Sciences 2021; 17(2): 71-3.
3. Prasetyo EP, Saraswati W, Goenharto S, Wahjuningrum D, Mooduto L, Rosidin RF, Tjendronegoro E. White pomegranate (*Punica granatum*) peels extract bactericidal potency on *Enterococcus faecalis*. Conservative Dentistry Journal 2021; 11(2): 84-8.
4. Soesilo D, Pangabdian F, Juniar E. Antibiofilm activity of *Cyanobacteria spirulina* as an irrigation solution against *Enterococcus faecalis*. Majalah Kedokteran Gigi Indonesia 2023; 9(1): 7-15.
5. Soesilo D, Puspita S, Christabel PF. Antibacterial effect of *Nannochloropsis oculate* as root canal sterilization material on *Streptococcus mutans* biofilm. Odonto Dental Journal 2021; 8(2): 119-25.
6. Harseno S, Mooduto L, Prasetyo EP. Antibacterial potency of kedondong Bangkok leaves extract (*Spondias dulcis* Forst.) against *Enterococcus faecalis* bacteria. Conservative Dentistry Journal 2016; 6(2): 110-6.
7. Cahyani F, Saraswati W, Prasetyo EP, Surboyo MDC, Riawan W, Saninggar KE, Hariyani N, Kusumawardani A, Soetojo A, Soetjipto. The effect of a-mangostin on TLR-4 and PGE2 in the dentin-pulp complex exposed to HEMA. Journal of International Dental and Medical Research 2022; 15(1): 21-6.
8. Santoso CMA, Samadi K, Prasetyo EP, Wahjuningrum DA. Root canal cleanliness between mangosteen peel extract irrigant and NaOCl 2.5%. Conservative Dentistry Journal 2020; 10(1): 40-3.
9. Dudeja PG, Dudeja KK, Srivastava D, Grover S. Microorganisms in periradicular tissues: do they exist? a perennial controversy. Journal of Oral and Maxillofacial Pathology. 2015; 19(3): 356-63.
10. Wong J, Manoil D, Nasman P, Belibasakis GN, Neelakantan P. Microbiological aspects of root canal infections and disinfection strategies: an update review on the current knowledge and challenges. Frontiers in Oral Health. 2021; 2:672887.
11. Ba-Hattab R, Al-Jamie M, Aldreib H, Alessa L, Alonazi M. Calcium hydroxide in endodontics: an overview. Open Journal of Stomatology. 2016; 6(12): 274-89.
12. Prasetyo EP, Widjiastuti I, Cahyani F, Kuntjoro M, Hendrijantini N, Hariyani N, Winoto ER, Nugraha AP, Goenharto S, Susilowati H, Hendrianto E, Rantam FA. Cytotoxicity of calcium hydroxide on human umbilical cord mesenchymal stem cells. Pesquisa Brasileira em Odontopediatria e Clinica Integrada 2020; 20: e0044.
13. Prasetyo EP, Kuntjoro M, Goenharto S, Juniarti DE, Cahyani F, Hendrijantini N, Nugraha AP, Hariyani N, Rantam FA. Calcium hydroxide increases human umbilical cord mesenchymal stem cells expressions of apoptotic protease-activating factor-1, caspase-3 and caspase-9. Clinical, Cosmetic and Investigational Dentistry 2021: 13: 59-65.
14. Prasetyo EP, Kuntjoro M, Cahyani F, Goenharto S, Saraswati W, Juniarti DE, Hendrijantini N, Hariyani N, Nugraha AP, Rantam FA. Calcium hydroxide upregulates Interleukin-10 expression in time dependent exposure and induces osteogenic differentiation of human umbilical cord mesenchymal stem cells. International Journal of Pharmaceutical Research 2021; 13(1): 140-5.
15. Sahara E, Farah RA, Zakaria MN. Calcium hydroxide as intracanal medicament in pulp necrosis with periapical lesion: a case report. Key Engineering Materials. 2019; 829: 226-31.
16. Putri CMD, Prisinda D, Malinda Y. The MIC and MBC of calcium hydroxide medicament against bacteria that cause a chronic periapical abscess in the vulnerable initial 7-days of endodontic treatment. Padjadjaran Journal of Dentistry. 2022; 34(1): 16-25.
17. Prasetyo EP, Juniarti DE, Sampoerno G, Wahjuningrum DA, Budi AT, Hasri D, Tjendronegoro E. The antibacterial efficacy of calcium hydroxide-iodophors and calcium hydroxide-barium sulfate root canal dressings on *Enterococcus faecalis* and *Porphyromonas gingivalis* in vitro. Dental Journal 2022; 55(2): 62-6.
18. Koral SM. Calcium oxide as a root filling material: a three-year prospective clinical outcome study. Open Dentistry Journal. 2011; 5:13-7.
19. Prakash V, Darsini J, Karthick A, Venkatesh A, Darsini H. Mineral Trioxide Aggregate (MTA)- an overview. European Journal of Molecular and Clinical Medicine 2020; 7(3): 2115-20.
20. Del M Saez M, Lopez GL, Atlas D, de la Casa ML. Evaluation of pH and calcium ion diffusion from calcium hydroxide pastes and MTA. Acta Odontologica Latinoameriacana. 2017; 30(1):26-32.
21. Mohammedi Z, Shalavi S, Yazdizadeh M. Antimicrobial activity of calcium hydroxide in endodontics: a review. Chonnam Medical Journal. 2012; 48(3): 133–40.
22. Kumar NK, Brigit B, Annapoorna BS, Naik SB, Merwade S, Rashmi K. Effect of triple antibiotic paste and calcium hydroxide on the rate of healing of periapical lesions: a systematic review. Journal of Conservative Dentistry 2021; 24(4): 307-13.
23. Kuntjoro M, Prasetyo EP, Cahyani F, Kamadjaja MJK, Hendrijantini N, Laksono H, Rahmania PN, Ariestania V, Nugraha AP, Ihsan IS, Dinaryanti A, Rantam FA. Lipopolysaccharide's cytotoxicity on human umbilical cord mesenchymal stem cells. Pesquisa Brasileira em Odontopediatria e Clinica Integrada 2020; 20: e0048.
24. Prasetyo EP, Sampoerno G, Juniarti DE, Cahyani F, Saraswati W, Kuntjoro M, Tjendronegoro E. Effect of lipopolysaccharide-induced apical periodontitis in diabetes mellitus rats on periapical inflammation. European Journal of Dentistry 2023; 17: 1146-52.
25. Momenijavid M, Salimizand H, Korani A, Dianat O, Nouri B, Ramazanzadeh R, Ahmadi A, Rostamipour J, Khosravi MR. Effect of calcium hydroxide on morphology and physicochemical properties of *Enterococcus faecalis* biofilm. Scientific Reports. 2022; 12: 7595.
26. Pushpalatha C, Dhareshwar V, Sowmya SV, Augustine D, Vinothkumar TS, Renugalakshmi A, Shaiban A, Kakti A, Bhandi SH, Dubey A, Rai AV, Patil S. Modified mineral trioxide aggregate - a versatile dental material: an insight on application and newer advancements. Frontiers in Bioengineering and Biotechnology. 2022; 10: 941826.
27. Malhan S, Mehta SD, Raji J. Mineral trioxide aggregate: a review. International Journal of Health Sciences 2021; 5(S1), 215-23.

28. Estrela C, Cintra LTA, Duarte MAH, Rossi-Fedele G, Gavini G, Sousa-Neto MD. Mechanism of action of bioactive endodontic materials. Brazilian Dental Journal. 2023; 34: 1–11.
29. Misra P, Bains R, Loomba K, Singh A, Sharma VP, Murthy RC, Kumar R. Measurement of pH and calcium ions release from different calcium hydroxide pastes at different intervals of time: atomic spectrophotometric analysis. Journal of Oral Biology and Craniofacial Research. 2017; 7(1): 36-41.
30. Li J, Xie S, Ahmed S, Wang F, Gu Y, Zhang C, Chai X, Wu Y, Cai J, Cheng G. Antimicrobial Activity and Resistance: Influencing Factors. Frontiers in Pharmacology. 2017; 8: 364.
31. Reygaert WC. An overview of the antimicrobial resistance mechanism of bacteria. AIMS Microbiology 2018; 4(3): 482–501.
32. Chellat MF, Raguz L, Riedl R. Targeting antibiotic resistance. Angewandte Chemie International Edition. 2016; 55(23): 6600–26.