Trad. Med. J., January-April 2020
Vol. 25(1), p 55-58
ISSN-p : 1410-5918 ISSN-e : 2406-9086

Submitted : 01-07-2019
Revised : 11-12-2019
Accepted : 02-04-2020

Evaluation of Antibacterial Potential of Carbonated Hydroxyapatite Combined with Propolis on *Porphyromonas gingivalis*

Nungky Devitaningtyas[1], Ahmad Syaify[2*], Dahlia Herawati[2], Suryono[2]
[1] Dentistry Program, Faculty of Dentistry Universitas Gadjah Mada, Yogyakarta, Indonesia
[2] Periodontology Department, Faculty of Dentistry, Universitas Gadjah Mada, Yogyakarta, Indonesia

ABSTRACT

Carbonated hydroxyapatite is ideal as a bone graft material because it has similar organic matters to the bone, excellent osteoconductive properties, and good biodegradation in the body. Hydroxyapatite contains the risk of being contaminated by bacteria called *Porphyromonas gingivalis (P. gingivalis)* in the oral cavity because it has no vascularization, therefore, facilitating adhesion of bacteria, and when applied in the oral cavity, it may cause an infection that then inhibits healing. Thus, it is necessary to use a material that has an antibacterial effect with low potential of causing resistance to treat the postsurgical infection properly. Propolis has antibacterial, antiviral, antifungal, antitumor, and immunomodulatory activities. Propolis contains a large number of flavonoids and phenols. The phenol compound in propolis is usually called caffeic acid phenethyl ester (CAPE), and it has a good antibacterial property. The study aims to evaluate the antibacterial effect of carbonated hydroxyapatite when immersed with different propolis concentrations of 2.5%, 5%, 7.5%, and 10% for 24 h and to measure the zone of inhibition against *P. gingivalis*. The Kruskal–Wallis test resulted in $p = 0.00$ ($p < 0.05$), indicating that there were significant differences among the test groups. The data processing was followed by Mann–Whitney *U*-test, and the results showed a significant difference in the group of carbonated hydroxyapatite-10 % propolis compared with the other groups. Inhibition zone of carbonated hydroxyapatite that immersed with propolis 10% showed the largest mean of diameters zone of inhibition.

Keywords: *Porphyromonas gingivalis*; Antibacterial; Carbonated hydroxyapatite; Propolis

INTRODUCTION

Guide tissue regeneration (GTR), also known as guide bone regeneration (GBR), is a therapy for periodontal tissue regeneration. This material shall be well biocompatible to the body, safe to use, nontoxic, and nonallergic, as well as contains no risk of disease transmission (Ana *et al.,* 2010). One of GBR or GTR materials is bone graft. Alloplastic bone graft is a material that can be either natural or synthetic and very easy to obtain, and it has a low risk of disease transmission, something that is frequently found in xenografts and allografts. One example of alloplastic material is carbonated hydroxyapatite (CHA; Landi *et al.,* 2003). CHA is ideal as a bone graft material because it has similar organic matters to the bone, excellent osteoconductive properties, and good biodegradation in the body (Dewi and Ana, 2018). However, surgical therapy using CHA contains the risk of being contaminated by bacteria in the oral cavity because it has no vascularization, thus facilitating adhesion of bacteria, and when applied in the oral cavity, it may cause infection, which then inhibits healing (Ardhani *et al.,* 2016). The bacteria that are responsible for infection in periodontal tissues are *Porphyromonas gingivalis (P. gingivalis)*. These are anaerobic gram-negative bacteria, and 85.75% can be found in subgingival plaques in patients with chronic periodontitis. The largest habitat of these bacteria is human gingival sulcus. These bacteria will survive through amino acid fermentation in the periodontal pocket, which has low sugar content. Virulence factors of *P. gingivalis* are found in fimbriae, LPS, proteases, outer membrane protein, and capsule (How *et al.,* 2016). In some cases, antibiotic prophylaxis is used to minimize postsurgical infections, but it may lead to resistance. Thus, it is necessary to use a material that has an antibacterial effect with low potential of causing resistance to treat the postsurgical infection properly.

Propolis is a substance that contains resinous, which is produced by honey bees to line the cells of a honeycomb that function as a defense system. Propolis has antibacterial, antiviral, antifungal, antitumor, and immunomodulatory activities (Kitamura *et al.*, 2018). Propolis contains a large amount of flavonoids and phenols. The phenol compound in propolis is usually called caffeic acid phenethyl ester (CAPE), and this has a good antibacterial property. CAPE can damage bacterial cytoplasmic membrane, inhibit nucleic acid synthesis, and increase the permeability of the

***Corresponding author : Ahmad Syaify**
Email : drgahmadsyaify@gmail.com

cell membrane, which leads to bacterial cell lysis (Bittencourt *et al.,* 2015).

A study conducted by Scatolini *et al*., (2018) revealed that hydroxyapatite material incorporated with dried propolis extract showed antibacterial activity against *Staphylococcus aureus* (*S. aureus*) bacteria. Therefore, this study incorporated CHA with propolis to see its antibacterial activity against *P. gingivalis* bacteria.

METHODOLOGY

Materials

Preparation of CHA

This study used 10 mg of GamaCha® CHA bone graft material.

Preparation of Propolis Solution

Propolis Brazilian® solution produced in Minas Gerais and distributed by Nusa Mega was used. The concentrations of the propolis solution were 2.5%, 5%, 7.5%, and 10%. A 10% propolis solution was obtained by mixing 45 mL of double distilled water with 5 mL of 10% propolis solution. Serial dilution was done in order to obtain 40 mL solution with 7.5% concentration, 30 mL solution with 5% concentration, and 20 mL solution with 2.5% concentration.

Preparation of CHA Incorporated in Propolis Solution

GamaCha® (10 mg) was immersed in 1.5 mL of each concentration of propolis solution. The immersion was done for 24 h at room temperature and then dried using an incubator at 37°C for 24 h. The material was then sterilized using ethylene oxide at RS Bethesda Yogyakarta.

Preparation of Bacteria

The *P. gingivalis* bacteria used were ATCC 33277 and available at the Microbiology Laboratory of the Faculty of Dentistry, Airlangga University Surabaya. The bacteria were prepared by cultivating them on brain heart infusion broth medium using osse wire and then incubated for 24 h. After that, the turbidity was adjusted to 0.5 McFarland turbidity standards, which is equal to 1.5×10^8 colony-forming units (CFU/mL). The test medium agar was made into five Petri dishes. The bacteria were developed in these media using swabs until they grew on the entire agar surface.

Method

Antibacterial Testing

The antibacterial property of CHA incorporated with propolis was done by measuring the diameter of bacterial growth inhibition against *P. gingivalis* bacteria through agar diffusion test. The procedure started by putting the test material on an agar and followed by observing whether the zone of inhibition was formed. If there were any zone of inhibition, the procedure would be continued by measuring the zone diameter using a Vernier caliper both horizontally and vertically. The observations were done after 24 h of incubation in millimeters. The measurement of the inhibition zone used the following formula:

$$Zone\ of\ Inhibition = \frac{(Dv - DS) + (Dh - Ds)}{2}$$

(Scatolini *et al.,* 2018)

Where Dv is the vertical diameter (in millimeters), Ds the diameter of wells (in millimeters), and Dh the horizontal diameter (in millimeters).

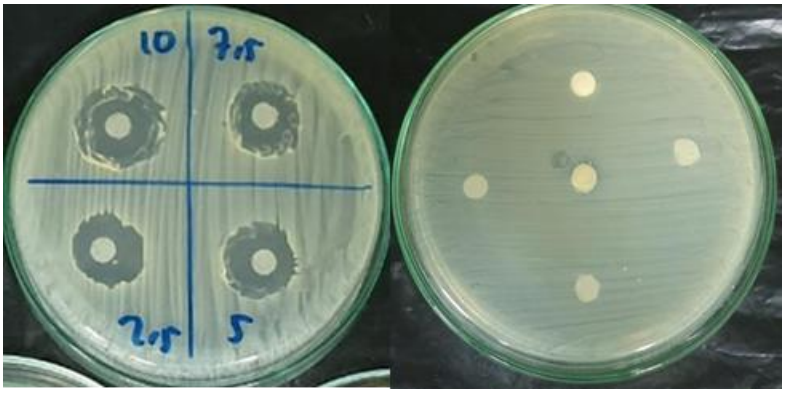
Statistical Analysis

All the data obtained from the observations were quantitative ratio data. The normality and homogeneity of bacterial inhibition zones were tested using Kruskal–Wallis continued with the Mann–Whitney U test. The significance value was set at $p < 0.05$.

RESULT AND DISCUSSION

The current study on the antibacterial evaluation of CHA material incorporated with propolis against the growth of *P. gingivalis* bacteria was divided into five treatment groups: CHA as negative control, CHA-2.5% propolis (CHA + 2.5% propolis), CHA-5% propolis (CHA + 5% propolis), CHA-7.5% propolis (CHA + 7.5% propolis), and CHA-10% propolis (CHA + 10% propolis), in five replicates (Figure 1). The measurements of the inhibition zones were carried out using a Vernier caliper in millimeters.

Based on the results of the statistical tests, it was found that CHA material incorporated with 10% propolis (CHA + 10% propolis) had the largest inhibition zone diameter compared with CHA incorporated with other concentrations of propolis (2.5%, 5%, and 7.5%; Figure 2). On the other hand, CHA as a negative control did not have antibacterial property. The Shapiro–Wilk normality test showed that the data were not normal, and the Levene homogeneity test showed that the data were not homogeneous because the data processing resulted in $p < 0.05$. The Kruskal–Wallis test resulted in $p = 0.00$ ($p < 0.05$), indicating that there were significant differences among the test groups. The data processing was followed by Mann–Whitney *U*-test, and the results showed a significant difference in the group of CHA + 10% propolis compared with the other groups.



(A) (B)

Figure 1. (A) Zone of inhibition in different concentration of 2.5%, 5%, 7.5%, and 1%; (B) Zone of inhibition of carbonated hydroxyapatite (negative control).

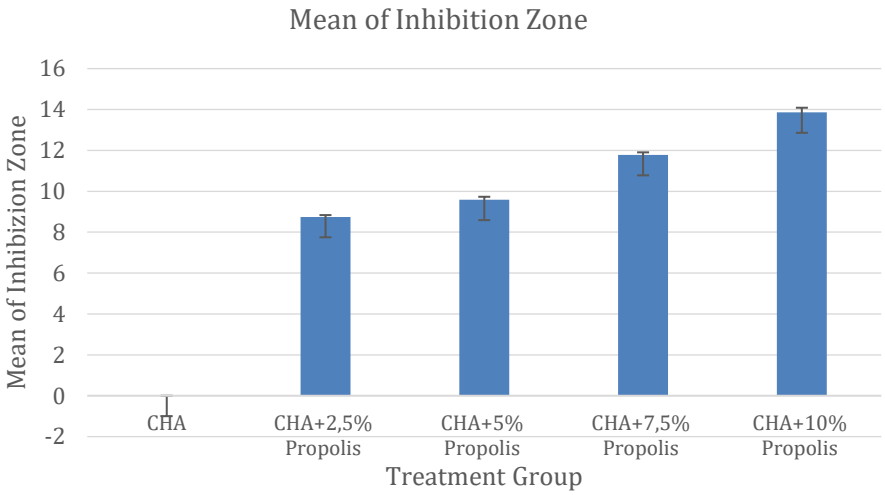


Figure 2. Mean of Inhibition Zone Diameters of *P. gingivalis*

This study combined CHA with propolis in order to analyze the ability of CHA to carry the antibacterial property of propolis. Immersing CHA in propolis solution for 24 h could produce a bactericidal effect against *P. gingivalis* bacteria. Scatolini *et al.,* (2018) conducted a study using hydroxyapatite incorporated with Brazilian propolis, showing that the mixture has bactericidal activity against *S. aureus* bacteria. Freires *et al.,* (2016) organized a study using mesoporous bioactive glass immersed in propolis solution for 24 h to inhibit the activities of *Enterococcus faecalis*, *S. aureus, Streptococcus mutans*, *Provotella intermedia, P. gingivalis, Aggregatibacter actinomycetemcomitans*, *and Fusobacterium nucleatum.*

The results of this study showed that CHA could carry the antibacterial property of propolis. It is proven from the clear zone formation around the well. The clear zones around the CHA wells immersed in a propolis solution with concentrations of 2.5%, 5%, 7.5%, and 10% showed that CHA could carry propolis as an antibacterial agent against *P. gingivalis* bacteria, whereas CHA as a negative control exhibited no clear zone formation. CHA immersed in 10% propolis solution had greater antibacterial activity compared with that immersed in concentrations of 2.5%, 5%, and 7.5%. The clear zone in CHA immersed in 10% propolis had a diameter of 13.86 mm. Propolis contains a variety of bioactive substances, including CAPE, which serves as an antibacterial agent. CAPE can damage bacterial cytoplasmic membrane, inhibit nucleic acid synthesis, and increase the permeability of the cell membrane, which leads to bacterial cell lysis (Al-Waili, 2018). CAPE inhibits RNA polymerase of

bacteria, which can degrade the cytoplasm membrane. This mechanism causes bacteria to lose potassium ion and lead bacteria to lysis. CAPE also has bactericidal activity by increasing membrane permeability of bacteria, which would cause loss of adenosine triphosphate and dysfunction of membrane transport, leading to bacterial death (Meyuhas *et al.,* 2015).

Antibacterial activity is influenced by the concentration of propolis solution during immersion. Lower concentration of propolis produces less antibacterial activity. A propolis solution with a concentration of 10%–30% has a high antibacterial activity against gram-positive and gram-negative bacteria, whereas the concentration of 80%–90% propolis solution has the lowest antibacterial ability (Ghasemi *et al.,* 2017). CHA immersed in 2.5% propolis solution had an inhibition zone diameter of 8.75 mm, whereas the one immersed in a 10% solution had an inhibition zone diameter of 13.86 mm.

CONCLUSION

The result shows that CHA incorporated with propolis through immersion method for 24 h had bactericidal activity against *P. gingivalis* bacteria. Propolis contains CAPE, a compound that can cause bacterial lysis by inhibiting nucleic acid synthesis, damaging bacterial cytoplasm membrane, and increasing bacterial permeability. Propolis with lower concentrations to immerse CHA produces less antibacterial activity. The highest antibacterial activity is found in CHA immersed in a 10% propolis solution.

ACKNOWLEDGMENT

This study was supported by grant Hibah Penelitian Dana Masyarakat Berbasis Luaran (Outcome based) Faculty of Dentistry Universitas Gadjah Mada, Republic of Indonesia, in the fiscal year 2019 under contract No. 4349/ UN1/ FKG1/ SetKG1/ PT/ 2019. The information appearing herein has been translated by a Center for International Language and Cultural Studies of Islamic University of Indonesia CILACS UII Jl. Demangan Baru No.24 Yogyakarta, Indonesia. Phone/Fax: 0274 540 255.

REFERENCES

Al-Waili, N., 2018, 'Mixing two different propolis samples potentiates their antimicrobial activity and wound healing property: A novel approach in wound healing and infection', *Vet. World* 11, 1188–1195.

Ana, I.D., Matsuya, S. & Ishikawa, K., 2010, 'Engineering of Carbonate Apatite Bone Substitute Based on Composition-Transformation of Gypsum and Calcium Hydroxide', *Engineering* 02, 344–352.

Ardhani, R., Setyaningsih, Hafiyyah, O.A. & Ana, I.D., 2016, 'Preparation of carbonated apatite membrane as metronidazole delivery system for periodontal application', *Key Engineering Materials* pp. 250–258.

Bittencourt, M.L.F., Ribeiro, P.R., Franco, R.L.P., Hilhorst, H.W.M., Castro, R.D. De & Fernandez, L.G., 2015, 'Metabolite profiling, antioxidant and antibacterial activities of Brazilian propolis: Use of correlation and multivariate analyses to identify potential bioactive compounds', *Food Res. Int.* 76, 449–457.

Dewi, A.H. & Ana, I.D., 2018, 'The use of hydroxyapatite bone substitute grafting for alveolar ridge preservation, sinus augmentation, and periodontal bone defect: A systematic review', *Heliyon* 4, e00884.

Freires, I.A., De Alencar, S.M. & Rosalen, P.L., 2016, 'A pharmacological perspective on the use of Brazilian Red Propolis and its isolated compounds against human diseases', *Eur. J. Med. Chem.*

Ghasemi, F.S., Eshraghi, S.S., Andalibi, F., Hooshyar, H., Kalantar- Neyestanaki, D., Samadi, A. & Fatahi-Bafghi, M., 2017, 'Anti-Bacterial Effect of Propolis Extract in Oil Against Different Bacteria', *Zahedan J. Res. Med. Sci.* 19, e7225. https://doi.org/10.5812/zjrms.7225.Y.,

Song, K.P. & Chan, K.G., 2016, 'Porphyromonas gingivalis: An overview of periodontopathic pathogen below the gum line', *Front. Microbiol.*

Kitamura, H., Saito, N., Fujimoto, J., Nakashima, K. ichi & Fujikura, D., 2018, 'Brazilian propolis ethanol extract and its component kaempferol induce myeloid-derived suppressor cells from macrophages of mice in vivo and in vitro', *BMC Complement. Altern. Med.* 18, 1–11.

Landi, E., Celotti, G., Logroscino, G., Tampieri, A., 2003, 'Carbonated hydroxyapatite as bone substitute', *J. Eur. Ceram. Soc.* 23, 2931–2937.

Meyuhas, S., Assali, M., Huleihil, M. & Huleihel, M., 2015, 'Antimicrobial activities of caffeic acid phenethyl ester', *J. Mol. Biochem.* 4, 21–31.

Scatolini, A.M., Pugine, S.M.P., De Oliveira Vercik, L.C., De Melo, M.P. & Da Silva Rigo, E.C., 2018, 'Evaluation of the antimicrobial activity and cytotoxic effect of hydroxyapatite containing Brazilian propolis', *Biomed. Mater.* 13, 0–31