Effectiveness of ChKM solution compared to triple-antibiotic paste as an intracanal medicament for bacteria that cause a chronic periapical abscess

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ABSTRACT

Introduction: Chronic periapical abscesses are caused by polymicrobial bacteria, including *E. faecalis*, *S. mutans*, *S. sanguinis*, and *P. gingivalis*. These bacteria can be eliminated with chlorophenol camphor menthol (ChKM) solution and triple-antibiotic paste (TAP) as an intracanal medicament. This study compared the effectiveness of ChKM solution to triple-antibiotic paste as an intracanal medicament for bacteria that cause a chronic periapical abscess. Methods: An experimental laboratory was conducted in-vitro with the microdilution method and optical density measurement using a spectrophotometer. The cell inhibition percentage was calculated to determine the MIC value. The MBC values were confirmed by cultivating the Mueller Hinton Agar samples, incubating them at 37°C for 24 hours, and observing bacterial growth. Bacteria did not grow in the medium at MBC value. The lower MIC and MBC were sensitive and could be an effective medicament choice. Results: The MIC ChKM solution inhibits *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, *S. sanguinis* ATCC 10556, and *P. gingivalis* ATCC 33277 were 4000, 4000, 2000, and 2000 µg/mL consecutively. While MIC of TAP were 6, 0.375, 0.75, and 1.5 µg/mL, respectively. Moreover, MBC of the ChKM solution were 32000, 32000, 8000, 8000 µg/mL and MBC of the TAP were 768, 24, 24, 96 µg/mL. Conclusions: ChKM solution and TAP effectively inhibited and killed *E. faecalis*, *S. mutans*, *S. sanguinis*, and *P. gingivalis* as an intracanal medicament, as seen from the MIC and MBC values. However, TAP is more effective than the ChKM solution because MIC and MBC values of TAP are much lower than the ChKM solution. This finding indicated that TAP is more effective at the lowest concentration than ChKM solution. It should be highlighted that this was an in-vitro study involving specific microbes; thus, further clinical research is needed.

Keywords: *E. faecalis*; *S. mutans*; *S. sanguinis*; *P. gingivalis*; ChKM solution; triple-antibiotic paste

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INTRODUCTION

A chronic periapical abscess is an inflammatory response originating from infection and pulp necrosis.\textsuperscript{1} The necrotic pulp is a source of pathogenic microorganisms and inflammatory mediators that gather to reach the periapical region through the apical foramen to stimulate the development of abscesses.\textsuperscript{2} Several studies have identified bacteria from the root canal associated with a periapical abscess caused by well-known microorganisms such as \textit{E. faecalis} (5\%), \textit{S. mutans} (10\%), \textit{S. sanguinis} (6.6\%), and \textit{P. gingivalis} (59\%).\textsuperscript{3,4} Bacteria play an essential role in developing pulp and periapical disease to become a control target in root canal treatment, one of the treatments for periapical abscesses.\textsuperscript{5}

Root canal treatment aims to control sepsis from the pulp and surrounding periapical tissue by removing root canal microorganisms and preventing re-infection. This treatment can only be achieved by preparing the root canal, disinfection, and obturation.\textsuperscript{6,7} Considering the complex anatomy of root canal space, most of the root canal wall is untouched if only by instrumentation alone at the preparation stage. Therefore, disinfection, including irrigation and intracanal medicament, is necessary to kill the remaining microorganism in the root canal.\textsuperscript{1,8}

One of the intracanal medicament materials often used in dentistry is the chlorophenol camphor menthol (ChKM) solution. ChKM solution is most widely used because it has the advantage of being able to spread. After all, it has a broad spectrum and is effective against bacteria. ChKM solution has strong disinfection activity. The admixture of menthol resulted in local anaesthesia and a particular anti-inflammatory effect, whereas the admixture of camphor is claimed to reduce the toxic effects of ChKM solution by reducing the water solubility of the phenol.\textsuperscript{9,10}

Nowadays, antibiotics as an intracanal medicament like triple-antibiotics paste (TAP) is commonly used. In Indonesia, TAP is known as 3mix. Because of the complexity of the root canal infection, it is unlikely that any single antibiotic could affect the canal's effective sterilisation. More likely, a combination would be needed to address the diverse flora encountered. The combination that appears to be most promising consists of metronidazole, ciprofloxacin, and minocycline.\textsuperscript{11,12} Besides TAP's ability to eradicate bacteria, some case reports indicate that minocycline can cause teeth discolouration. Lately, clinical studies have reported success in replacing minocycline with clindamycin. Both have a similar mechanism of action.\textsuperscript{13,14}

Both ChKM solution and TAP are made up of several ingredients. TAP is more beneficial than the ChKM solution for periapical abscesses treatment since they do not require a combination of other intracanal medicaments. TAP as an intracanal medicament is a gentle treatment that requires almost no instrumentation.\textsuperscript{12} The authors wanted to compare the efficacy of ChKM solution to TAP with clindamycin. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) in the microdilution method can determine the antibacterial activity of intracanal medicament. The dilution concentration is measured as optical density by a spectrophotometer at a specific wavelength.\textsuperscript{15} This study compared the effectiveness of ChKM solution to triple-antibiotic paste as an intracanal medicament for bacteria that cause a chronic periapical abscess.

METHODS

The present study was an \textit{in-vitro} laboratory experiment. The tool used in this study was a spectrophotometer with a wavelength of 600 nm. The materials used in this study were ChKM solution, metronidazole, ciprofloxacin, clindamycin in powder, macrogol, propylene glycol, sterile distilled water, Mueller-Hinton broth, Mueller-Hinton agar, \textit{E. faecalis} ATCC 29212, \textit{S. mutans} ATCC 25175, \textit{S. sanguinis} ATCC 10556 and \textit{P. gingivalis} ATCC 33277 obtained from the Integrated Laboratory of Universitas Padjadjaran, Jatinangor.

512 µL ChKM solution and 488 µL DMSO 2% were combined in a microtube and mixed until homogenous to obtain an initial concentration of 512000 µg/mL ChKM solution. Meanwhile, tri antibiotic paste was made by grinding the tablets of 500mg metronidazole, 200mg ciprofloxacin, and 300mg clindamycin into powder and mixed until thoroughly homogenised. The three antibiotics powders were mixed with macrogol
liquid and propylene glycol in a ratio of 7:1 to form a homogeneous paste and then put into a sterile syringe. The prepared paste was weighed and diluted in 1 mL of distilled water, then homogenised with a vortex to achieve a 1536 g/mL concentration.

The MIC test determination was performed with the microdilution method. Bacteria were made suspensions in the Mueller-Hinton Broth (MHB) medium, and McFarland 0.5 standard was used. The ChKM solution was diluted by 12 times serial dilution. The three types of antibiotics were mixed with macrogol and propylene glycol in a ratio of 1:7 to form a paste, then 1536 mg of paste was diluted using 1 mL of distilled water to obtain a concentration of 1.536 µg/mL. MHB media of 100 µL were placed on all well-microplates using a micropipette.

The 12 times serial dilution concentrations of ChKM solution are 256000, 128000, 64000, 32000, 16000, 8000, 4000, 2000, 1000, 500, 250, and 125 µg/mL while the TAP concentration are 768, 384, 192, 96, 48, 24, 12, 6, 3, 1.5, 0.75, and 0.375 µg/mL. The controlled variables and solvent used in this study were 2% DMSO. The microplate was sealed with parafilm paper and incubated for 18-24 hours at 37°C. The microplate was removed from the incubator to measure Optical Density (OD) using a spectrophotometer. Optical density values were used in the formula for cell inhibition percentage to get the MIC value. The MIC value was the lowest concentration of medicament, which was still able to inhibit bacterial growth. The percentage of cell inhibition was obtained from the reduction between the optical density of solvent and bacterial media. Then divided by the optical density of the bacterial media, and multiplied by one hundred per cent.

Concentrations that have been determined as MIC to the highest concentration were then planted on the media to visually observe bacterial growth gradation after being incubated for 18-24 hours at 37°C. Bacteria did not grow in the medium at MBC value. Therefore, the lower MIC and MBC were defined as sensitive and could be an effective medicament choice.

RESULTS

The percentage inhibition value of ChKM solution against bacteria is presented in Figure 1. The most significant percentage increase was determined as the MIC value. The percentage inhibition of ChKM solution against *E. faecalis* based on Figure 1 began to be seen at a 4000 µg/mL concentration. This condition was caused by a very significant percentage increase between the concentration of 4000 µg/mL to 8000 µg/mL. Therefore, the MIC value of ChKM solution against *E. faecalis* was 4000 µg/mL, *S. mutans* was 4000 µg/mL, *S. sanguinis* was 2000 µg/mL, and *P. gingivalis* was 2000 µg/mL.

The percentage inhibition value of TAP solution against bacteria is presented in Figure 2. The MIC value of TAP against *E. faecalis* was 6 µg/mL, *S. mutans* was 0.375 µg/mL, *S. sanguinis* was 0.75 µg/mL, and *P. gingivalis* was 1.5 µg/mL.
The obtained MIC was then planted on Mueller Hinton Agar (MHA) media, starting from the concentration selected as MIC to the highest concentration. After incubating for 24 hours at 37°, the bacteria-killing activity can be seen. The researcher calculated the MBC value twice to guarantee that the data obtained were accurate. The line border areas are made to mark the site of bacterial growth after incubation.

The confirmed observations for the MBC for ChKM solution (Figure 3) showed no growth of *E. faecalis* in the first and second trials of MHA starting at a concentration of 32000 µg/mL. Observations on MHA showed no growth of *S. mutans* in the first and second trials starting at a concentration of 32000 µg/mL (Figure 4). Observations on MHA showed no growth of *S. sanguinis* in the first and second trials starting at a concentration of 8000 µg/mL (Figure 5). Observations on MHA showed no growth of *P. gingivalis* in the first and second trials, starting at a concentration of 8000 µg/mL (Figure 6).
The confirmed observations for the MBC of the TAP on MHA plates on the first and second plantings (Figure 7) began to show no growth of *E. faecalis* at a concentration of 768 μg/mL. Observation of the MHA plates on the first and second repetitions (Figure 8) began to show no growth of *S. mutans* at a 24 μg/mL concentration. Observation of the MHA plates on the first and second repetitions (Figure 9) began to show no growth of *S. sanguinis* at a 24 μg/mL concentration. Observation of the MHA plates on the first and second repetitions (Figure 10) began to show no growth of *P. gingivalis* at a 96 μg/mL concentration.

**Figure 5.** Observations of *S. sanguinis* at a concentration of 4000 µg/mL still showed bacterial growth, while at a concentration of 8000 µg/mL did not show any bacterial growth: (A) and (B) The first trial of ChKM solution against *S. sanguinis*; (C) and (D) The second trial.

**Figure 6.** Observations of *P. gingivalis* at a concentration of 4000 µg/mL still showed bacterial growth, while at a concentration of 8000 µg/mL did not show any bacterial growth: (A) and (B) The first trial of ChKM solution against *P. gingivalis*; (C) and (D) The second trial.

**Figure 7.** Observations of *E. faecalis* at a 768 μg/mL concentration showed no bacterial growth: (A) The first trial of TAP against *E. faecalis*; (B) The second trial.

**Figure 8.** Observations of *S. mutans* at a 24 μg/mL concentration showed no bacterial growth: (A) and (B) The first trial of TAP against *S. mutans*; (C) and (D) The second trial.
Recapitulation of MIC and MBC values of chlorophenol camphor solution and triple-antibiotic paste against *E. faecalis* ATCC 29212, *S. mutans* ATCC 25175, *S. sanguinis* ATCC 10556, and *P. gingivalis* ATCC 33277 of the present study is presented in Table 1.

Table 1. Recapitulation of MIC and MBC values against *E. faecalis*, *S. mutans*, *S. sanguinis*, and *P. gingivalis*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC (µg/mL)</th>
<th>MBC (µg/mL)</th>
<th>TAP</th>
<th>MIC (µg/mL)</th>
<th>MBC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecalis</em> ATCC 29212</td>
<td>4000</td>
<td>32000</td>
<td>6</td>
<td>768</td>
<td></td>
</tr>
<tr>
<td><em>S. mutans</em> ATCC 25175</td>
<td>4000</td>
<td>32000</td>
<td>0.375</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td><em>S. sanguinis</em> ATCC 10556</td>
<td>2000</td>
<td>8000</td>
<td>0.75</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td><em>P. gingivalis</em> ATCC 33277</td>
<td>2000</td>
<td>8000</td>
<td>1.5</td>
<td>96</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The bacteria used in this study follow the bacteria reported by Nobrega et al.\(^3\) and Sousa et al.\(^4\) as bacteria that cause periapical abscesses, including *S. sanguinis*, *S. mutans*, *P. gingivalis*, and *E. faecalis*.\(^5,6\) Those bacteria are available in ATCC preparations, cultured first in the Mueller-Hinton Broth (MHB) media. MHB media is recommended as the media of choice for antibacterial testing because it supports satisfactory growth for most pathogens, especially when evaluating the MIC for an antibacterial agent.\(^7\)

The study results showed that ChKM solution had antibacterial effectiveness against *E. faecalis* ATCC 29212 at a 4000 µg/mL concentration. This result aligns with research conducted by Setiawan et al.\(^18\), which showed an antibacterial effect at a 2000 µg/mL concentration. The difference in the effectiveness of concentration in the two studies could be due to the different methods used. A ChKM solution is a phenol group that has been shown to inhibit the growth of *E. faecalis* bacteria. ChKM solution has a higher antibacterial, antiseptic and disinfectant potential than other disinfectants or phenol. It is based on its ability to destroy the bacteria membrane by binding on its proteins and lipids.\(^9,18\)

The results show that ChKM solution has antibacterial activity against Gram-positive and Gram-negative bacteria such as *Streptococcus* spp., *E. faecalis*, and *P. gingivalis*. Based on Table 1, it can be seen that ChKM solution has bacteriostatic activity against *S. sanguinis* and
that may occur at concentrations of 2000 μg/mL and bactericidal activity at a minimum concentration of 8000 μg/mL. These results align with the research conducted by Prijatmoko et al., who stated that ChKM solution has antibacterial activity against Gram-positive bacteria such as *E. faecalis* and *Streptococcus spp.*. The method is the differences between the studies conducted by Prijatmoko et al. and the current research. The current research used the microdilution method, while Prijatmoko et al. used the disc diffusion method. In the study using the disc diffusion method, it was seen that ChKM solution produced an inhibition zone of 16-17 mm. Antibacterial activity is weak if the inhibition zone diameter is <8 mm, moderate is 8-14 mm, strong is 15-19 mm, and very strong if >20 mm, so it can be concluded that ChKM solution has a strong antibacterial activity against Gram-positive bacteria.

ChKM solution is included in the derivatives of phenol compounds. The mechanism of action of phenol compounds in inhibiting bacterial growth, namely by denaturing bacterial cell proteins, inhibits cell membrane function (transporting substances from one cell to another) and inhibits nucleic acid synthesis so that bacterial growth can be inhibited. The mechanisms of action of phenolic compounds on bacterial cells have been partially attributed to damage to the bacterial membrane, inhibition of virulence factors such as enzymes and toxins, and suppression of bacterial biofilm formation.

The results show that TAP has antibacterial effectiveness against *E. faecalis*. These results align with the research of Khoshkhounejad et al., who stated that TAP with clindamycin modification can inhibit and kill *E. faecalis*. A study conducted by Sabrah et al. showed that at a concentration of 300 μg/mL, the TAP consisting of metronidazole, ciprofloxacin, and minocycline showed bactericidal activity against *E. faecalis*.

The study conducted by Nalawade et al. using the agar diffusion method stated the MIC of an antibiotic paste against *S. mutans* ATCC 25175 at a concentration of 1.95 μg/mL, where the antibiotic paste used is a combination of metronidazole and ciprofloxacin. Another study conducted by Sabrah et al. using the dilution method through optical density measurements showed the TAP MIC against *P. gingivalis* at a 6 μg/mL concentration. Moreover, the MBC value at a concentration of 300 μg/mL, where the TAP used, consisted of metronidazole, ciprofloxacin, and minocycline.

Metronidazole in the TAP has antibacterial activity against many anaerobic bacteria, both Gram-positive and Gram-negative such as *E. faecalis*, *S. mutans*, *S. sanguinis*, and *P. gingivalis*. Metronidazole is almost always bactericidal, which can quickly enter the bacterial cell membrane. Metronidazole attaches itself to bacterial DNA when dealing with bacterial DNA and then destroys the helical structure to inhibit bacterial DNA replication and fragmentation, which quickly leads to cell death in bacteria.

Clindamycin in this study was effective against Gram-positive and Gram-negative anaerobes such as *E. faecalis*, *S. mutans*, *S. sanguinis*, and *P. gingivalis*. The mechanism of action of clindamycin is to bind exclusively to the 50S bacterial ribosomal subunit to suppress bacterial protein synthesis. These bonds result in inhibition of the binding of aminoacyl-transfer ribonucleic acid (tRNA) or can result in dissociation of peptidyl tRNA from the bacterial ribosome.

Ciprofloxacin also has extreme antibacterial activity against Gram-negative bacteria such as *P. gingivalis* but is limited to Gram-positive bacteria. Ciprofloxacin works in inhibiting the action of the enzyme DNA gyrase and topoisomerase IV. In bacterial replication and transcription, the double helix form of DNA from bacteria must be separated into two strands of DNA. This separation results in overwinding or positive supercoiling of the double helix DNA. DNA gyrase enzymes in bacteria have a function to overcome this by creating negative supercoiling. If the work of the DNA gyrase enzyme is inhibited, the obstacles in the form of positive supercoiling are not overcome so that the process of bacterial replication and transcription becomes disrupted.

The American Association of Endodontists has recommended using TAP not to exceed 1000 μg/mL, although there is no suggestion of proper concentration in use as an intracanal medicament. That statement is consistent with research results in this study, where the resulting concentration does not exceed 1000 μg/mL. However, limitations in this study include selecting limited and less extensive concentrations, so it
cannot be known whether there is a bacteriostatic
effect lower than the concentration tested. In addition, the numerical number is not the
only factor to consider when comparing MICs of
different intracanal medicaments but depends on
the distance between the MIC and the breakpoint,
the infection site, and other factors like the ages,
health, and bacterial species. The medicament’s adverse effects and the frequency and route of
administration are all significant considerations.

CONCLUSIONS

ChKM solution and TAP effectively inhibited and
killed E. faecalis, S. mutans, S. sanguinis, and P.
gingivalis as an intracanal medicament, determined
from the MIC and MBC values. However, TAP is
more effective than the ChKM solution because TAP’s MIC and MBC values are much lower than the
ChKM solution. This result indicates that the TAP is
effective at a lower concentration than the ChKM
solution. It should be highlighted that this is an in-vitro study involving specific microbes; thus,
further clinical research is needed.

REFERENCES


