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Comparison of 3% Mepivacaine and 2% Procaine in Local Anesthetics as Antibacterial Activity on the Growth of Porphyromonas Gingivalis

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Abstract

In dentistry, local anesthesia is needed, especially for oral and maxillofacial surgery to reduce the sensation of pain. Besides its use as an analgesic, local anesthetics also have antibacterial activity which inhibits the growth of various bacteria.

The purpose of this study was to determine the antibacterial activity of procaine and mepivacaine in inhibiting the growth of Porphyromonas gingivalis.

In this study, the antibacterial activity test was carried out using the Kirby-Bauer diffusion method for ten repetitions and four treatment groups tested, 2% procaine, 3% mepivacaine, metronidazole as a positive control, and aquadest as a negative control dripped on paper discs and incubated for 24 hours. The results showed that procaine and mepivacaine had antibacterial activity with inhibition zone diameters of 9.36 mm and 8.47 mm, metronidazole 11.80 mm, and aquadest showed no inhibition diameter.

The results of the research data were analyzed using the One-way ANOVA test. There was a significant difference in the diameter of the inhibition zone ($p < 0.05$) between the four treatment.

Two percent procaine and 3% mepivacaine have antibacterial activity against the growth of Porphyromonas gingivalis which is classified as a moderate inhibitory response according to David and Stout.

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Introduction

In dentistry, local anesthetics are needed, especially in oral and maxillofacial surgery.[1] Local Anesthetics can be administered topically or given by injection to reduce pain sensation and as a pain control.[2] Local anesthetics are indicated for various dental procedures such as tooth extraction, apicoectomy, gingivectomy, gingivoplasty, periodontal surgery, pulpectomy, pulpotomy, alveoplasty, dental implants, jaw fracture treatment, avulsion tooth reimplantation, pericoronitis, cysts, tumor surgery, suturing, flapping of mucoperiosteum tissue.[3] These are invasive procedures that are prone to post-treatment infections that can result in delayed wound healing due to pathogenic bacterial activity.[4] The human oral cavity has the most complex microorganism flora in the body, consisting of 6 billion bacteria representing more than 700 species with nine different phyla. In one of the phyla, namely the Bacteroidetes phylum, there is one of the normal microorganisms that can be found in the oral cavity, namely the bacterium Porphyromonas gingivalis.[5]

However, these bacteria can turn into pathogens if there is a decrease in the function of the body's defense system and an increase in the number of bacteria, which causes the balance of interaction between the host and bacteria in the oral cavity to be disturbed.[6] Porphyromonas gingivalis is a bacterium that plays a role in the development of periodontal disease and is also known to inhibit wound healing (delayed wound healing) after invasive actions/surgical procedures by inhibiting epithelial cell migration.[4] To prevent this, prophylactic antibiotics are generally recommended.[7] However, if antibiotics are not

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used appropriately, it can make the disease difficult to treat, inhibit the healing process, and inappropriate use can also risk allergic reactions.[7,8]

In addition to the use of antibiotics as the main choice in the treatment of bacterial infections, several studies have shown that local anesthetics also have an antibacterial role that may support the use of antibiotics as an additional antibacterial. The antibacterial activity of local anesthetics was first discovered by Jonnesco in 1909.[2] Local anesthetics are drugs that function to reduce the sensation of temporary pain in certain parts of the body. Local anesthetics are divided into two groups, namely, ester group and amide group.[9,10] Ester group local anesthetics consist of cocaine, benzocaine, ametocaine, procaine, tetracaine, chloroprocaine. Amide group local anesthetics consist of Articaine, Bupivacaine, Dibucaine, Etidocaine, Levobuvipacaine, Lidocaine, Mepivacaine, Prilocaine, Ropivacaine, Sameridine, Tonicaine, Cinchocaine.[9,10]

One of the amide group local anesthetics known in dentistry is mepivacaine. Mepivacaine is the third most commonly used local anesthetic after lidocaine and articain. Mepivacaine is indicated for local infiltration anesthesia, nerve block, and epidural and has similar characteristics to lidocaine, namely short-acting and rapid onset. Mepivacaine exhibits a milder vasodilating ability that results in a longer duration of anesthesia even without a vasoconstrictor.[11,12] Through several studies, mepivacaine not only functions as an analgesic, but can also function as an antibacterial. Research on the antibacterial effect of local anesthetic mepivacaine has been started since 1996 on several bacteria. Mepivacaine became one of the local anesthetics studied against the growth of Porphyromonas gingivalis bacteria because mepivacaine is quite often used in dental practice after the use of lidocaine and articaine which have been studied for their antibacterial activity against the growth of Porphyromonas gingivalis bacteria. Procaine is a pharmaceutical drug that belongs to the ester class of local anesthetics that have a slow onset and short duration of action. Procaine and other ester group local anesthetics are commonly used for infiltration anesthesia, peripheral nerve blocks, and spinal blocks.[13] Procaine is one of the ester group local anesthetics that is often used in dentistry and is safer than cocaine. Procaine not only has analgesic effects, but also has antibacterial effects with research showing the potential for antibacterial activity on procaine. Research on the antibacterial effect of procaine has been started since 1971 but only with some gram-positive bacteria, while research on gram-negative bacteria is still very little. Proven by clinical doses, procaine showed an inhibitory effect on the growth of some of these bacteria.[14,15]

The mechanism of antibacterial action of local anesthetics occurs through growth inhibition, decreased cell viability, destruction of protoplasts, changes in membrane permeability leading to leakage of intracellular components, and inhibition of cell membrane-dependent enzymatic activity. These factors allow the antibacterial activity of mepivacaine and procaine local anesthetics.[14,15] The aim of this study is to open the possibility of the potential use of local anesthetic mepivacaine or procaine as an additional antibacterial to reduce the growth of one of the bacteria namely Porphyromonas gingivalis, in addition to prophylactic antibiotics to prevent infection and delayed wound healing due to invasive / surgical actions in dental treatments.

Materials and methods

This type of research is pure experimental research using the Kirby-Bauer diffusion method (disc diffusion test) and with post test only control group research design. The test samples used were Porphyromonas gingivalis bacteria obtained from Aretha medika main laboratory in Bandung and there were four treatment groups using metronidazole solution as a positive control which is a gold standard antibiotic in the treatment of diseases caused by anaerobic bacteria, distilled water as a negative control, 2% procaine and 3% mepivacaine as the experimental group.

All tools and materials that will be used in this study must be washed, dried and sterilized to be in a sterile condition to avoid the presence of compounds or microorganisms that can affect the process and results of the study. Tools such as ose are sterilized by heating the tool over a bunsen flame with repetition two to three times. Non-precision glass tools such as erlemeyer, baker glass, petri dish are sterilized by oven heating at 160-180°C for 1.5-3 hours. The tools

are wrapped first using paper before sterilization. Tools such as microbiological media, micropipettes, measuring cups, cotton swabs, volumetric flasks were sterilized by autoclaving at 121°C for 15 minutes. Then wait until all tools reach room temperature and dry.[16,17,18]

Muller Hinton Agar as much as 38 grams was dissolved in 1000 ml of distilled water in an Erlenmeyer tube and covered with cotton. The solution was heated using a microwave until boiling and homogeneous. After the solution is homogeneous, sterilize it in an autoclave at 121°C with a pressure of 1.5 atm for 20 minutes, then put it in a Petri dish and leave it to harden

Muller Hinton Broth (MHB) as much as 21 grams was dissolved in 1000 ml of distilled water in an Erlenmeyer tube and using microwave assistance so that the solution was homogeneous. After that, sterilization was carried out using an autoclave at a temperature of 121°C with a pressure of 1.5 atm for 20 minutes. Then take a few ml of Muller hinton broth and put it into a test tube that will be used in the preparation of Porphyromonas gingivalis bacterial inoculum.

The ose needle is sterilized over a bunsen flame 2-3 times and then left until the ose is cold. Insert the sterile ose into the Porphyromonas gingivalis (ATCC 33277) bacterial culture and take the isolated bacterial culture colonies and place them on Muller Hinton agar media and then incubate for 24 hours at 37°C.[16,18]

Bacterial identification is carried out to obtain pure culture after bacterial isolation. Pure bacterial cultures are used to determine the type of bacteria by looking at their morphology, properties, and biochemical abilities.[18,19] Identifying bacteria is done by observing macroscopic characteristics. Macroscopically, it is done by observing the shape of the colony, the size of the colony, the margin or edge of the colony, the surface of the colony, and the smoothness of the surface.[18,19] Porphyromonas gingivalis bacteria macroscopically have a pleomorphic colony shape of short rods (coccobacilli), produce black-brownish color pigments (black pigmented), colony size 0.5 µm x 1-2 µm, smooth colony surface, convex, shiny, and smooth edges.[16,19]

Preparation of bacterial inoculum is done by direct colony suspension method. Inoculum was obtained by inoculating Porphyromonas gingivalis colonies that had been cultured for 18-24 hours on Muller Hinton Agar (MHA), into Muller Hinton Broth (MHB). Bacterial suspensions that have been incubated, take colonies from Muller Hinton agar using an ose needle that has been sterilized over a bunsen flame. Transfer the colonies to a tube containing muller hinton broth until the turbidity reaches the McFarland 0.5 standard to obtain an inoculum with a bacterial count of approximately 1-2×10^8 CFU/mL.D[20] The suspension is taken using a sterile cotton swab. The cotton swab is pressed against the tube wall to remove excess suspension, then applied to the surface of Muller Hinton agar (MHA) evenly (swab method) and allowed to stand at room temperature with the cup closed for 3 to 5 minutes until the suspension is absorbed into the agar.[16,19] A 3% concentration of mepivacaine hcl, 2% concentration of procaine hcl, and 500mg/100 ml injection metronidazole liquid was poured into a test tube

The test method used is the Kirby and Bauer disc diffusion method using a 6 mm disc paper that has been dripped with 3% mepivacaine hcl local anesthetic, 2% procaine hcl, metronidazole solution and distilled water solution. Then the disc paper was placed on a Petri dish containing agar media that had been planted with Porphyromonas gingivalis bacteria and incubated in an incubator at 37°C for 24 hours.[16,17,19] After 24 hours, observe the inhibition zone formed around the disc paper, then take measurements using a caliper.

Inhibition zone measurements were taken after the incubation process was complete. Measurements were made using a caliper to measure the diameter of the clear zone/inhibition zone formed.[21,22] The inhibition zone measured was the horizontal inhibition zone and the vertical inhibition zone.[18,21,23] The inhibition zone that has been measured is then adjusted to the classification of the inhibition response according to David and Stout which is divided into 4, ≤ 5 mm weak, 5-10 mm medium, 10-20 mm strong, ≥ 20 mm very strong.[24, 25]

Formula: $L = \frac{(D1-D3) + (D2-D3)}{2}$

Description:
L = Width of zone of inhibition.
D1 = Diameter of horizontal zone of inhibition.
D2 = Diameter of vertical zone of inhibition.
D3 = Diameter of paper disk.

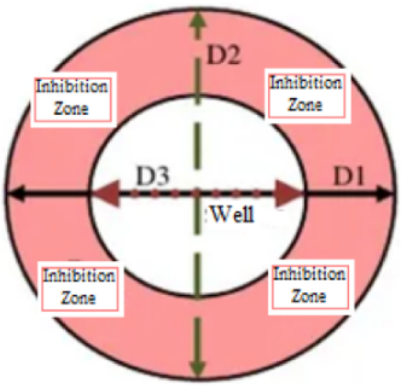

Figure 1. How to measure the diameter of bacterial inhibition zone[18,21].

Data from the results of the study will be presented in the form of computerized tables. Data will be analyzed by univariate analysis to assess mean and standard deviation, as well as bivariate analysis to assess normality test and hypothesis testing. The data normality test uses the Shapiro-Wilk test to determine the distribution of data with sample of less than 50. Data distribution is said to be normal if the p value> 0.05, but if the p value <0.05, then the data is not normally distributed. The data obtained from the research results are not normally distributed with a p value <0.05, so data transformation is carried out in logarithmic form which is then tested again to assess data normality. After transforming the data and testing again, it was found that the data was normally distributed with a p value >0.05, so it could be continued with the One-way ANOVA hypothesis test. The hypothesis is meaningful if the p value is <0.05. Then it will be continued with the LSD (*Least Significant Difference*) post hoc test.

Results

Research on the antibacterial activity of local anesthetic 2% procaine and 3% mepivacaine in inhibiting the growth of Porphyromonas gingivalis bacteria was conducted in vitro. There were four treatment groups using metronidazole solution as a positive control which is a gold standard antibiotic in the treatment of diseases caused by anaerobic bacteria, distilled water as a negative control, 2% procaine and 3% mepivacaine. The test was carried out using nutrient agar media, namely Muller Hinton agar, then placed paper disks that had been tested. Next, the samples were incubated and observed to see the inhibition zone or clear zone formed around the disc paper after 24 hours. Each group was carried out nine times with one repetition as a drop out sample to prevent failure in repetition, with observations made of all repetitions from each group at the same time

Testing the antibacterial activity of local anesthetics 2% procaine and 3% mepivacaine against the growth of Porphyromonas gingivalis bacteria was carried out using the Kirby-Bauer diffusion method (disc diffusion test), by measuring the inhibition zone formed around the disc paper. The test was conducted using 2% procaine, 3% mepivacaine, metronidazole as positive control, and distilled water as negative control. The results of measuring the diameter of the inhibition zone can be seen in the table below.

No.	Inhibition zone diameter (mm)	Treatment			
		Metronidazole (mm)	Distilled water (mm)	*2% Procaine* (mm)	*3% Mepivacaine* (mm)
1.	Repetition 1	10.58	0	9.10	8.21
2.	Repetition 2	11.09	0	8.78	8.53
3.	Repetition 3	12.76	0	10.01	8.05
4.	Repetition 4	13.02	0	7.96	10.16
5.	Repetition 5	13.05	0	10.11	8.13
6.	Repetition 6	11.87	0	9.43	8.19
7.	Repetition 7	10.01	0	9.87	8.22
8.	Repetition 8	11.21	0	9.21	8.35
9.	Repetition 9	12.58	0	9.76	8.42
Average		**11.80**	**0**	**9.36**	**8.47**

Table 1. Measurement results of inhibition zone diameter of 2% procaine, 3% mepivacaine, metronidazole, distilled water against the growth of Porphyromonas gingivalis bacteria.

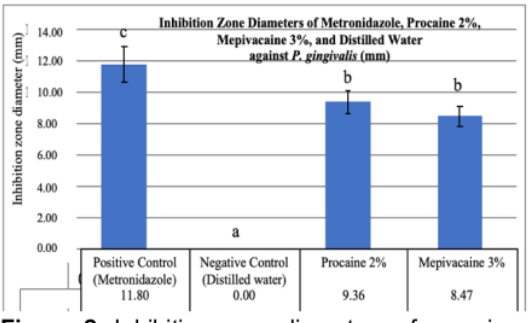

Figure 2. Inhibition zone diameters of procaine, mepivacaine, metronidazole, and distilled water.

Based on the measurement results above, it was found that the average diameter of the inhibition zone on procaine 2% was 9.36 mm, mepivacaine 3% was 8.47 mm, the positive control, metronidazole, was 11.80 mm, and the negative group, distilled water, was 0 mm. Data on the diameter of the inhibition zone of 2% procaine, 3% mepivacaine, metronidazole, and distilled water were then tested for normality using the ShapiroWilk test because the study sample was less than 50 samples and to determine whether the data was normally distributed or not.

No.	Treatment Group	Repetitions	P-*Value*
1.	*Metronidazole*	9	0.341
2.	Akuades	9	-
3.	2% *Procaine*	9	0.396
4.	3% *Mepivacaine*	9	0.000

Table 2. Normality Test Results with Shapiro-Wilk Test.

Based on the results of the normality test above, the p value of metronidazole, is 0.341, 2% procaine is 0.396, and 3% mepivacaine is 0.000. Data distribution is said to be normal if the p value> 0.05, but if the p value <0.05 , then the data is not normally distributed, so based on the results above, the data are not normally distributed so that data transformation is carried out in logarithmic form and then tested again for normality.

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
logmetro	0.204	9	.200*	0.912	9	0.332
logprocaine	0.165	9	.200*	0.905	9	0.280
logmepivacaine	0.338	9	.075	0.628	9	0.204

Table 3. Data Normality Test Results in Logarithmic Form.

Based on the results of the normality test above, it is found that all data are normally distributed with a p value> 0.05 so that it can be continued with the Oneway ANOVA test. After testing the normality of the data using the Shapiro-Wilk test, the data was found to be normally distributed. Next, the data will be analyzed using the One-way Anova test to test whether there is a significant difference between the four treatment groups, namely metronidazole, distilled water, 2% procaine, and 3% mepivacaine.

No	Treatment Group	Repetitions	Average Zone of Inhibition Diameter (mm)	Standard Deviation	p-*Value Anova*
1.	*Metronidazole*	9	11.80	1.13	
2.	Distilled water	9	0	0.00	
3.	2% *Procaine*	9	9.36	0.69	P= 0.000
4.	3% *Mepivacaine*	9	8.47	0.65	

Table 4. One-way Anova Test Results.

The results of the One-way Anova test showed a p value <0,05, which means that the inhibitory power between the positive control, namely metronidazole, negative control, namely distilled water, and other treatment groups has a meaningful or significant difference in inhibiting the growth of Porphyromonas gingivalis bacteria. Furthermore, to determine the comparison of the diameter of the inhibition zone between treatment groups, the One-way Anova test results were continued with the LSD (Least Significant Difference) post hoc test. Based on the results of the LSD (Least Significant Difference) post hoc test, when each group is compared with each other, it has a meaningful or significant difference ($p < 0.05$).

	Metronidazole	Distilled water	2% *Procaine*	3% *Mepivacaine*
Metronidazole	-	0.000	0.000	0.000
Distilled Water	0.000	-	0.000	0.000
2% *Procaine*	0.000	0.000	-	0.006
3% *Mepivacaine*	0.000	0.000	0.006	-

Table 5. LSD (Least Significant Difference) post hoc test results (p-value).

	Metronidazole	Distilled Water	2% *Procaine*	3% *Mepivacaine*
Metronidazole	-	1.06996	.09983	.14294
Distilled Water	-1.06996	-	-.97013	-.92703
2% *Procaine*	-.09983	.97013	-	.04311
3% *Mepivacaine*	-.14294	.92703	-.04311	-

Table 6. LSD (Least Significant Difference) post hoc test results (mean difference).

Discussion

Based on the results of the research conducted, 2% procaine and 3% mepivacaine local anesthetics have antibacterial activity. The results of the test are in the form of observations of the inhibition zone or clear zone formed around the disc paper that has been dripped by 2% procaine and 3% mepivacaine after incubation for 24 hours at 37°C. Based on the

results obtained, it can be seen that there is an inhibition zone formed around the discs that are tetesi 2% procaine and 3% mepivacaine. This indicates a significant inhibition of Porphyromonas gingivalis bacterial growth from 2% procaine and 3% mepivacaine local anesthetics.

Two percent procaine has an average inhibition zone diameter of 9.36 mm and 3% mepivacaine has an average inhibition zone diameter of 8.47 mm. In addition, the positive control group, namely metronidazole, has an average inhibition zone diameter of 11.80 mm and the negative control group, namely distilled water, has no inhibition against the growth of Porphyromonas gingivalis bacteria which is characterized by the absence of an inhibition zone around the disc paper dabbed with distilled water. Based on David and Stout's classification, in this study, metronidazole had a strong inhibition response that ranged from 10-20 mm, while 2% procaine and 3% mepivacaine local anesthetics both had a moderate inhibition response although 2% procaine showed a larger inhibition zone diameter compared to 3% mepivacaine.

The difference in inhibition diameter could be because procaine has a better ability to affect DNA production and protein synthesis of bacteria because procaine not only works on the surface membrane, but also on the internal membrane which then reduces mechanical activity so that bacterial growth will be more inhibited in procaine compared to mepivacaine.[18,26] The difference in the diameter of the inhibition zone is also very likely caused by several factors such as The lower the pH, the less growth there will be, The sensitivity of organisms, culture media, incubation conditions, agar diffusion speed which influenced by microorganism concentration, media composition, incubation temperature, and incubation time during the research process that has been carried out, Density of the bacterium.[18,26,27] Different densities in each treated petri dish can affect the difference in the results of the diameter of the inhibition zone.[18,25] In addition, a low density in the petri dish will not cause the formation and growth of lawn growth on the agar medium, and a high density will not allow the development of an accurate zone of inhibition, Moisture and certain medium components such as thymine, sulfonamides, or thymidine can inhibit the activity of antibiotics such as trimethoprim, and may favor one of the treatment groups due to their content.[18,26,27]

In 1909, research was first conducted on the antibacterial activity test of local anesthetics which then over time continued to develop regarding this type of research.[2] In 1996 to 1998, research conducted by Sakuragi showed the level and potential of antibacterial activity of local anesthetics depending on dose, time, and temperature.[2] Previous research conducted by Kesici, et al. in 2019 using Staphylococcus aureus and Escherichia coli bacteria against lidocaine.[28] Based on the results of his research, it was found that lidocaine was able to inhibit the growth of Staphylococcus aureus bacteria with an inhibition zone diameter of 12 mm. In Escherichia coli, the diameter of the inhibition zone produced was 15.60 mm.[28]

There was another previous study also conducted by Klaus Pelz who used Staphylococcus aureus bacteria and also used seven types of local anesthetics commonly used in dentistry, namely lidocaine, articaine, bupivacaine, butanilicaine, procaine, mepivacaine, and prilocaine.[2,28] In this study, it was proven that local anesthetics have different inhibitory power against the growth of Staphylococcus aureus bacteria. Local anesthetic articaine had an average inhibition of 9.0 mm, bupivacaine of 2.0 mm, prilocaine of 9.3 mm, lidocaine 7.0 mm, butanilicaine of 6.7 mm, mepivacaine of 8.0 mm and procaine above 8 mm.[28] A comparative study of the antibacterial effects of several local anesthetics such as articaine, bupivacaine, mepivacaine, prilocaine, lidocaine, butanilicaine, and procaine in combination with four preservatives and vasoconstrictor components showed different minimal inhibitory concentrations and minimal bactericidal concentrations. Through this comparative study, antibacterial activity was shown to originate from the local anesthetic component, and not from the preservative or vasoconstrictor components.[2,28]

Procaine and mepivacaine are both local anesthetics but come from different classes where procaine is an ester class local anesthetic, while mepivacaine is an amide class local anesthetic.[3] A number of hypotheses have been proposed to explain the antibacterial activity in local anesthetics. Both local anesthetics have an antibacterial role as bacteriostatic or inhibit bacterial growth.[3,11] The longer the exposure time

of local anesthetics to bacteria, the greater the inhibition of bacterial growth.[3,11] Bacterial growth can be inhibited by local anesthetics resulting from damage to the bacterial cell wall or cytoplasmic membrane, leakage of intracellular components, inhibition of dehydrogenase activity, and increased cell wall permeability.[3,11,22]

The penetration of the bacterial membrane results from the electron bonding of the local anesthetic molecule to the polar bond related to the hydrophobic nature of the local anesthetic on the surface of the cell membrane. Local anesthetics can inhibit the activity of cell respiration membrane and change its permeability and solubility, thus causing leakage of cytoplasmic components characterized by the release of metal ions that play an important role in cell metabolism, and also inhibit membrane protein synthesis by causing an increase in lipid molecules, resulting in changes in membrane fluidity in the process of selective protein.[2,3,11,22] The presence of antibacterial compounds, in this case procaine and mepivacaine, on the cell surface can change the physical and chemical properties of the membrane so that it will inhibit the transport process of substances needed by the cell. This can interfere with the growth and kill bacterial cells. Damage to the cell wall which acts as a shape-giving structure in cells that protect cells from lysis and osmosis can cause bacterial cell lysis.[11,17,22]

This study proved that 2% procaine and 3% mepivacaine local anesthetics have antibacterial effects in inhibiting the growth of Porphyromonas gingivalis bacteria in vitro where 2% procaine has greater inhibition power because it shows a larger diameter of the inhibition zone compared to 3% mepivacaine. This suggests that the benefits of local anesthetics procaine and mepivacaine may extend beyond their analgesic effects in dentistry, opening up the potential use of local anesthetics as antibacterial adjuncts in invasive dental procedures. Based on the research results, it can be concluded that 2% procaine and 3% mepivacaine has antibacterial activity that can inhibit the growth of Porphyromonas gingivalis. There are differences in the antibacterial activity of 2% procaine and 3% mepivacaine. Two percent Procaine has a greater inhibition ability when viewed from the diameter of the inhibition zone formed which is 9.36 mm compared to 3% mepivacaine with an inhibition zone diameter of 8.47 mm, although both are categorized as having moderate inhibition according to David and Stout classification.

Conclusions

Two percent procaine and 3% mepivacaine have antibacterial activity against the growth of *Porphyromonas gingivalis* which is classified as a moderate inhibitory response according to David and Stout.

Declaration of Interest

The authors report no conflict of interest.

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