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In vitro evaluation of the antimicrobial activity of CTZ paste and its components on standard microorganisms

Farli Aparecida Carrilho Boer[1], *Lucas Fernando Oliveira Tomáz Ferraresso*[1], *Karina Tomoe Ajimura*[1], *Regina Lúcia dos Santos*[2], *Wanda Terezinha Garbelini Frossard*[3].

Abstract: CTZ paste (chloramphenicol, tetracycline, zinc oxide and eugenol) is used to treat deciduous molars with necrotic pulp. The antimicrobial activity (AA) of different concentrations of the components of the CTZ paste against different microorganisms was evaluated *in vitro*. Thus, the medications were divided into 6 groups: P1: 250 mg of tetracycline + 250 mg of chloramphenicol + 500 mg of zinc oxide (ZO) + 0.6 ml of eugenol (original proportion), P2: 500 mg of tetracycline + 500 mg of ZO + 0.6 ml eugenol, P3: 500 mg chloramphenicol + 500 mg ZO + 0.6 ml eugenol, P4: 1000 mg ZO + 0.6 ml eugenol, P5: 0.006 ml eugenol, P6: 1000 mg of ZO + saline solution (0.85%), and evaluated against individual standard microorganisms: *Streptococcus mutans*, *Lactobacillus casei*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Bacillus subtilis*. Agar diffusion tests (ADT) and direct exposure tests (DET) were applied. The data were subjected to ANOVA and Tukey test ($p<5$) were applied. P1 had greater AA compared to the other groups. For the evaluation of *Escherichia coli*, no statistically significant difference was observed between groups P1 and P3. Direct exposure test demonstrated antimicrobial effectiveness for groups P1, P2, P3, P4 and P5 in the time intervals evaluated and ineffectiveness for P6 due to the absence of eugenol. Therefore, it can be concluded that group P1 presented the highest antimicrobial activity among groups in the ADT. DET demonstrated antimicrobial effectiveness in groups with eugenol in its composition, which suggests a bactericidal effect of this component.

Key words: Anti-Infective Agents, Dental Caries, Eugenol, Root Canal Filling Materials, Zinc Oxide.

Evaluación *in vitro* de la actividad antimicrobiana de la pasta CTZ y sus componentes sobre microorganismos estándar

Resumen: La pasta CTZ (cloranfenicol, tetraciclina, óxido de zinc y eugenol) se utiliza para tratar los molares decíduos con pulpa necrótica. Se evaluó *in vitro* la actividad antimicrobiana (AA) de diferentes concentraciones de los componentes de la pasta CTZ contra diferentes microorganismos. Los medicamentos se dividieron en 6 grupos: P1: 250 mg de tetraciclina + 250 mg de cloranfenicol + 500 mg de óxido de zinc (OZ) + 0,6 ml de eugenol (proporción original), P2: 500 mg de tetraciclina + 500 mg de OZ + 0,6 ml de eugenol, P3: 500 mg de cloranfenicol + 500 mg de OZ + 0,6 ml de eugenol, P4: 1000 mg de OZ + 0,6 ml de eugenol, P5: 0,006 ml de eugenol, P6: 1000 mg de OZ + solución salina (0,85%) y evaluado contra microorganismos estándar individualmente: *Streptococcus mutans*, *Lactobacillus casei*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Bacillus subtilis*. Se aplicaron pruebas de difusión en agar (PDA) y exposición directa (PED). Los datos fueron sometidos a ANOVA y prueba de Tukey ($p<5$). El grupo P1 tenía mayor AA en comparación con otros grupos. Para *Escherichia coli*, no se observó diferencia estadísticamente significativa entre los grupos P1 y P3. PED demostró AA para los grupos P1, P2, P3, P4 y P5 en los intervalos de tiempo evaluados e ineficacia para P6 debido a la ausencia de eugenol. El grupo P1 presentó el AA más alto entre los grupos del PDA. PED demostró AA en grupos con eugenol en su composición, lo que sugiere efecto bactericida.

Palabras clave: Antiinfecciosos, Caries Dental, Eugenol, Materiales de Obturación del Conducto Radicular, Óxido de Zinc.

[1]*Clínica de Especialidades infantiles/ Bebê Clínica de la Universidad Estadual de Londrina. Brasil.*
[2] *Universidade Estadual de Londrina. Brasil.*

Avaliação *in vitro* da atividade antimicrobiana da pasta CTZ e seus componentes sobre microrganismos padrões

Resumo: A pasta CTZ (cloranfenicol, tetraciclina, óxido de zinco e eugenol) é utilizada para o tratamento de molares decíduos com polpa necrótica. Avaliou-se *in vitro* a atividade antimicrobiana (AA) de diferentes concentrações dos componentes da pasta CTZ contra diferentes microorganismos. Os medicamentos foram divididos em 6 grupos: P1: 250 mg de tetraciclina + 250 mg de cloranfenicol + 500 mg de óxido de zinco (OZ) + 0,6 ml de eugenol (proporção original), P2: 500 mg de tetraciclina + 500 mg de OZ + 0,6 ml de eugenol, P3: 500 mg de cloranfenicol + 500 mg de OZ + 0,6 ml de eugenol, P4: 1000 mg de OZ + 0,6 ml de eugenol, P5: 0,006 ml de eugenol, P6: 1000 mg de OZ + solução salina (0.85%), e avaliados contra microrganismos padrões individualmente: *Streptococcus mutans*, *Lactobacillus casei*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Bacillus subtilis.* Utilizou-se testes de difusão em ágar (TDA) e exposição direta (TED). Os dados foram submetidos à ANOVA e teste de Tukey ($p<5$). O grupo P1 teve maior AA comparativamente aos demais grupos. Para a avaliação da *Escherichia coli*, não observou diferença estatisticamente significativa entre os grupos P1 e P3. TED demonstrou efetividade antimicrobiana para os grupos P1, P2, P3, P4 e P5 nos intervalos de tempo avaliados e inefetividade do P6 devido a ausência do eugenol. Conclui-se que o grupo P1 apresentou no TDA, a maior AA entre grupos. TED demonstrou AA nos grupos com eugenol em sua composição, o que sugere efeito bactericida desse componente.

Palavras-chave: Anti-Infecciosos; Cárie Dentária; Eugenol; Materiais Restauradores do Canal Radicular; Óxido de Zinco.

Introduction

Pulp therapies in deciduous teeth represent procedures commonly performed in pediatric dentistry that aim to maintain the integrity, health and functionality of teeth and supporting tissues until their natural exfoliation[1]. Pulp therapies can be divided into conservative and invasive, and diagnosis is essential for selecting the type of treatment to be performed[2,3].

In cases of pulp necrosis in deciduous molars, challenges are imposed on the operator due to the anatomical and morphological complexity of these teeth, characterized by the presence of accessory canals, curvatures, great microbial diversity and irregular rhizolysis[4-6]. These factors make effective chemical-mechanical preparation and reliable establishment of apical limits and difficult, and these difficulties have a toll on the cooperation of young children[4,6]. The choice and use of an antimicrobial paste for obturation that has a broad spectrum of action, good diffusion and that does not cause damage to the periapical tissues and permanent teeth, represents one of the most important aspects for the success of pulp therapy[7,8].

Although there is no consensus in the literature regarding the ideal paste to use for deciduous teeth with pulp necrosis, there are several antimicrobial filling materials available, such as: calcium hydroxide paste, zinc oxide and eugenol[1], iodoform paste[1] and CTZ paste (chloramphenicol, tetracycline, and zinc oxide - proportion 1:1:2 - associated with eugenol)[10,11]. This paste was proposed by Cappiello in 1964, in Argentina, for the treatment of deciduous teeth with necrotic pulp and can be used for the techniques of Lesion Sterilization and Tissue Repair (LSTR) or root canal filling[10,12,13]. The properties of

this endodontic material include a broad spectrum of action, high bacteriostatic activity[10,11,14] with effects on bacterial protein synthesis and inhibition of bacterial growth[10,11], and satisfactory clinical and radiographic results[14]. CTZ paste can be found commercially in specialized compounding laboratories in the form of capsules, with the requested proportion. From a clinical point of view, the powder should be dispensed onto a sterile glass plate and mixed with a drop of eugenol using a sterile metal spatula, until it acquires a consistency similar to toothpaste. Furthermore, the procedure for inserting the paste is simple, does not require instrumentation or additional root canal filling steps[10,11], can be performed in one session[10,14,15], promotes the stabilization of bone resorption, and does not cause tissue sensitivity[14,15]. Among the advantages of using this material are the reduced clinical time, the child's cooperation and the optimization of time spent caring for the child[13,14].

Considering the scarce literature on the use of CTZ paste in deciduous teeth with pulp necrosis, the present study aimed to evaluate in vitro the antimicrobial activity and efficacy of the CTZ paste components, used individually or in different combinations and concentrations against six microorganisms previously selected for Diffusion Test and direct exposure test.

Methodology

Study design - first stage

All these strains were isolated, identified, cultivated, and maintained viable in the laboratory of State University of Londrina, Londrina, Paraná, Brazil. To carry out the tests in the first and second stages, the CTZ paste and its components were divided into six groups of medicines according Table 1. The antimicrobial activity of each group was evaluated individually against six standard microorganisms of different species from the American Type Culture Collection (ATCC), morphologically distinct, with a predominance of facultative anaerobic

Table 1. *Composition of the six groups of medicines evaluated.*

Groups	Antibiotics	Zinc oxide	Saline solution (0.85%)	Eugenol
P1	250 mg de tetraciclina + 250 mg de cloranfenicol	500 mg	———————	0,6 ml
P2	500 mg de tetraciclina	500 mg	———————	0,6 ml
P3	500 mg de cloranfenicol	500 mg	———————	0,6 ml
P4	———————————	1000 mg	———————	0,6 ml
P5	———————————	———————	———————	0,006 ml
P6	———————————	1000 mg	X	———————

bacteria (Table 2). These microorganisms were selected because they are commonly found in cases of pulp necrosis in deciduous teeth[7,16,17].

The liquid and solid culture media used are described in table 3. The preparation and sterilization process to which they were subjected (121°C/15 min) were carried out in accordance with the manufacturer's instructions.

The standard microorganisms, which were lyophilized or in stock agar, were initially recovered in 3 ml of BHI broth or specific medium and incubated at 37°C/24h. The suspension obtained was adjusted to tube #1 of the McFarland Scale, until obtaining a final microbial concentration of approximately 3 X 108 bacteria/ml, considered ideal for the development of the research. From this sample, a volume of 0.1 ml of this suspension (inoculum) was sown in six tubes with 3 ml of BHI broth or specific medium, for each standard microorganism evaluated.

Table 2. *Microorganisms used in the study.*

Standard microorganisms	Abbreviations	Characteristics
Streptococcus mutans (INCQS 0054-CCT 3440) (ATCC 25175)	M1	Gram-positive, non-sporulating, facultative anaerobic cocci. They are predominantly found in the oral cavity and act as an etiological agent of tooth decay.
Lactobacillus casei (CT) INCQS 0006 (ATCC 7469)	M2	Gram-positive, non-sporulating, facultative anaerobic (sometimes microaerophilic) rods. They are found in the oral cavity and play an important role in the etiology of tooth decay.
Staphylococcus aureus (INCQS 00039)	M3	Gram-positive, non-sporulating cocci, facultative anaerobes, present in infections such as trauma, dental abscesses, pharyngitis, tonsillitis, sinusitis and osteomyelitis of the face.
Enterococcus faecalis (ATCC 23212)	M4	Gram-positive, non-sporulating, facultative anaerobic cocci. They are found in low concentrations in the oral cavity and may be associated with infectious processes, especially in dental root canals.
Escherichia coli (INCQS 00032)	M5	Gram-negative, non-sporulating rods; aerobes or facultative anaerobes. Its natural habitat is the intestinal tract of humans and animals.
Bacillus subtilis (CT) INCQS 000349 (ATCC 9372)	M6	Gram-positive, aerobic sporulating bacilli. They consist of saprophytic microorganisms that prevail in soil, water, air and vegetation.

Table 3. *List of culture media selected for the cultivation of different types of standard microorganisms.*

Culture mediums	Standard microorganisms
BHI broth (Brain Heart Infusion- Acumedia®)	*Escherichia coli; Enterococcus faecalis; Staphylococcus aureus; Bacillus subtilis*
BHI Agar (Brain Heart Infusion- Acumedia®)	*Escherichia coli; Enterococcus faecalis; Staphylococcus aureus; Bacillus subtilis*
Mitis salivarius broth*	*Streptococcus mutans*
Mitis salivarius broth (Acumedia®)	*Streptococcus mutans*
Tomato juice broth*	*Lactobacillus casei*
Tomato juice agar (Acumedia®)	*Lactobacillus casei*

** *Mitis salivarius* broth and tomato juice broth were manipulated.

The sowing technique chosen to carry out the agar diffusion test was the "pour-plate". Standard microorganisms were evaluated individually in all experiments carried out. A volume of 0.1 ml of the microbial suspension, containing 20 ml of BHI agar or specific medium, dissolved at a temperature of 40-45°C, was inoculated into 18 tubes for each standard microorganism. Subsequently, the media were poured into Petri dishes and subjected to gentle rotational movements to mix the microbial suspension (inoculum) in the agar, in order to obtain confluent growth of colonies in the culture medium. To evaluate the M5 group, it was necessary to adjust the inoculum volume from 0.1 ml to 5 ml in 20 ml of mitis salivarius agar.

After sowing the plates, five cavities were created in the agar, 4mm in diameter and 4mm deep (thickness corresponding to the layer of culture medium in question), with the aid of a sterilized metal perforator, aiming to deposit the groups of pastes in a standardized.

The pastes were obtained by homogenizing the powder components (Laboratory OdontoFarma, Londrina, Paraná, Brazil), on a glass plate with a 24F metal spatula (SSWhite Duflex, Juiz de Fora, MG, Brazil), both sterilized, followed by incorporation into eugenol (Biodinâmica Química-Farmacêutica Ltda, Ibiporã, PR, Brazil). These were spatulated for 1 minute, until they acquired the consistency of toothpaste.

Groups P1, P2, P3, P4 and P5 were deposited in the previously prepared cavities, with the help of sterile wooden sticks, until they were filled. To evaluate eugenol, corresponding to group P6, it was carried out on filter paper discs, 6 mm in diameter, deposited under the agar with the aid of clinical cotton tweezers 317 (Golgran, São Caetano do Sul, São Paulo, Brazil). In this experiment, a negative control was performed for each standard microorganism.

After inserting the pastes into the wells, the plates were pre-incubated for 1 hour at room temperature, followed by incubation in a bacteriological oven at 37°C, for a period of 24 to 48 hours, according to the standard microorganism evaluated. The experiments were carried out aseptically and in triplicate. In total, 108 Petri dishes were evaluated.

Sample evaluation and statistical analysis

After this period, the results were analyzed by measuring the diameters of the microbial growth inhibition halos, formed around each cavity, with the aid of a millimeter ruler (Tilibra®, Bauru, São Paulo, Brazil) and a good source of reflected light. Only the diameters corresponding to the sharpest edges of the halos were noted.

The data obtained in the evaluation of each standard microorganism were subjected to statistical analysis using Analysis of Variance (ANOVA) and Tukey's test with a significance level of 5% (IBM SPSS Statistics for Windows Software, version 25 (IBM, Armonk, NY , USA).

Second stage

In the second stage, the effectiveness of the antimicrobial action of these groups

was carried out using the direct exposure test. This test consisted of immersing paper cones contaminated with standard microorganisms in Petri dishes containing the evaluated medicines, where they remained in direct contact with them for 5 min, 10 min, 15 min, 30 min, 1 h and 24 h. After this interval, the cones were individually inserted into 5ml tubes of BHI or specific media and incubated at 37°C/120h. The results were interpreted by observing the turbidity in the culture medium.

Results

From the results of the diameters of the halos, in millimeters, arising from the period of 24 and 48 hours, it can be seen that group P1 (corresponding to the complete paste), presented the highest values (in mm) of the average diameters of the halos of inhibition, compared to the five standard microorganisms evaluated in the experiment. In descending order, groups P3, P2, P4, P5 and P6 presented the largest inhibition halos (Figure 1).

Considering that the purpose of this study was not to make a comparison between the microorganisms evaluated, but only between the groups of medicines, it was found that the results outlined in this graph differed little between the microorganisms. The P1 group presented, in all experiments, greater antimicrobial activity in relation to the other groups, with the exception of the evaluation of Escherichia coli, in which there was no statistically significant difference between the means of the P1 and P3 groups. The same observation was made for the means of groups P4 and P5, which did not show a significant difference (5%) in all standard microorganisms evaluated.

Tables 4 and 5 describe the results obtained in evaluating the effectiveness of the antimicrobial action of the 6 groups of medicines against standard microorganisms, through direct exposure, in periods of 5min, 10min, 15min, 30min,

Figure 1. *Graph of the average diameters of the inhibition zones presented by the five standard microorganisms evaluated.*

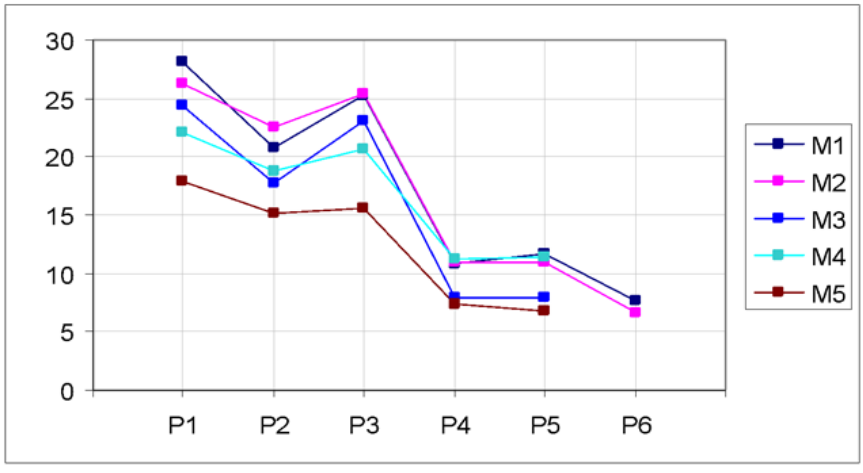

Table 4. *Representation of the results obtained in the direct exposure test for the drug groups: P1, P2, P3, P4 and P5, at different time intervals.*

Standard microorganisms	Time intervals					
	5 min	10 min	15 min	30 min	1 h	24 h
Staphylococcus aureus	---	---	---	---	---	---
Enterococcus faecalis	---	---	---	---	---	---
Escherichia coli	---	---	---	---	---	---
Bacillus subtilis	---	---	---	---	---	---
Streptococcus mutans	---	---	---	---	---	---
Lactobacillus casei	---	---	---	---	---	---
Endodontic paste activity	BE	BE	BE	BE	BE	BE

(-) Negative result = Absence of turbidity = Absence of microbial growth = Effectiveness of the medicine.
(+) Positive result = Presence of turbidity = Presence of microbial growth = Ineffectiveness of the medication.
(BE) = Bactericidal effect.
(---) = The signs represent the triplicate evaluation of the paper points.

Table 5. *Direct exposure test applied to P6 (Zinc Oxide and Saline Solution) at different time intervals.*

Standard microorganisms	Time intervals					
	5 min	10 min	15 min	30 min	1 h	24 h
Staphylococcus aureus	+++	+++	+++	+++	+++	+++
Enterococcus faecalis	+++	+++	+++	+++	+++	+++
Escherichia coli	+++	+++	+++	+++	+++	+++
Bacillus subtilis	+++	+++	+++	+++	+++	+++
Streptococcus mutans	+++	+++	+++	+++	+++	+++
Lactobacillus casei	+++	+++	+++	+++	+++	+++
Endodontic paste activity	IN	IN	IN	IN	IN	IN

(-) Negative result = Absence of turbidity = Absence of microbial growth = Effectiveness of the medicine.
(+) Positive result = Presence of turbidity = Presence of microbial growth = Ineffectiveness of the medication.
(IN) = Ineffectiveness of the medication in the evaluated time interval.
(+++) = The signs represent the triplicate evaluation of the paper points.

1h, 24h. The control groups in this experiment were not expressed in the tables. The negative control, represented by test tubes containing only sterile broth, showed no turbidity in the culture medium. The positive control indicated the presence of turbidity in the 24-hour reading for all standard microorganisms evaluated. The analysis of this result was macroscopic and microscopic, using the Gram Staining Method (GSM). The results presented in the tables correspond to the 120h reading of the experiment.

Discussion

The present study aimed to evaluate both isolated and combined components of CTZ paste against different standard microorganisms, and which had the greatest impacts. The high prevalence of untreated dental caries and dental trauma in deciduous teeth often can progress to irreversible pulp damage, leading to abscesses and fistulas. In cases of pulp necrosis, polymicrobial infection of the root canals typically shows a predominance of *Streptococcus* and anaerobic microorganisms[18,19]. This fact supports the use of the selected microorganisms in the present study, based on their characteristics outlined in Table 2. In this context, selecting a paste such as CTZ, which contains two antibiotics, is a key factor for achieving success in the proposed treatment. Additionally, it is essential for the clinician to understand its characteristics, limitations, antimicrobial effect, as well as the clinical convenience that CTZ paste provides.

It is known that microbial growth inhibition halos express the diffusion capacity of the drug evaluated in solid culture medium and the size depends on the solubility and diffusibility of the tested drug. For this reason, halos may not effectively express the full potential of the antimicrobial agent[20,21]. The speed of diffusion in agar and the size of the growth inhibition zone depend on factors such as: agar concentration, temperature, pH, nutritional characteristics, ion concentrations in the culture medium, thickness of the agar layer and inoculum concentration[20,21]. In order to minimize potential interference with the results, the procedures were performed by two operators, aseptically and in triplicate.

A consideration of this study refers to the evaluation of the antimicrobial activity of CTZ paste and its components on *Lactobacillus casei* using the Agar diffusion test. One of the difficulties encountered in these experiments was the application of the pour-plate sowing technique in this evaluation. It was observed in 6 plates sown with a volume of 1 ml of inoculum in 20 ml of tomato juice agar, confluent growth of bacterial colonies in the agar, after 48h of incubation in a bacteriological oven at 37°C. However, the formation of inhibition halos or diffusion halos around the cavities filled with the test drug was not observed in these results. The reason why this prevented the free diffusion of the medicine under the agar was not clarified in this study. Confirmation of the presence of microbial growth was carried out through microscopic observation of the culture medium, using GSM as a parameter.

Thus, it was chosen to perform sowing by spreading. Using this technique, a volume of 0.1 ml of inoculum was distributed over the entire surface of the tomato agar, with the aid of a Drigalski loop. After the incubation period corresponding to 48 hours, the formation of halos around the evaluated drugs was observed. These halos were not completely translucent, as is often observed in zones of microbial growth inhibition. Although the tomato juice broth was carefully handled by the operators to prepare the microbial suspension, the existence of tomato pulp residues was also observed, which, deposited on the agar, made it difficult to interpret the results in the internal region of the halos formed.

Therefore, it was then necessary to verify the presence of bacterial colonies in this region by collecting a portion of this residue located close to the evaluated medicine, with the aid of a platinum loop in a dragging movement. This material was stained by GSM and analyzed microscopically. The investigation was carried out on three plates, for the 6 groups of medicines. As the entire experiment was carried out in triplicate, 18 slides were evaluated, confirming the presence of colonies of the tested microorganism inside the halos.

Based on these observations, six paper discs containing the antimicrobials tetracycline (30 µg) and chloramphenicol (30 µg) were evaluated, with the aim of knowing the susceptibility of these antimicrobials (dissociated from the paste) on *Lactobacillus casei*, by the disk-diffusion method. The experiment was performed in three Petri dishes, sown by spreading on tomato agar, with a volume of 0.1 ml of inoculum. The discs containing the antibiotics to be tested (3 for each antimicrobial) were deposited on the agar and incubated for 48 h at 37°C, following the reading and measurement of the inhibition zones with a millimeter ruler, which was compared with the measurements provided by the record producers.

Through this test, it was observed that Lactobacillus casei were resistant to chloramphenicol and intermediately sensitive to tetracycline. The presence of colonies within the halo formed around the cavities filled with the medicine may be related to the resistance presented by this microorganism to the antimicrobials evaluated. Furthermore, tetracycline and chloramphenicol, when combined, gave the paste greater antimicrobial activity in relation to its components evaluated separately, as proposed by Cappiello (1964)[10]. The presence of synergism in this interaction between two bacteriostatic antibiotics was observed for all microorganisms evaluated, with the exception of the evaluation of *Escherichia coli*, in which there was no significant difference between the means of groups P1 and P3. The data collected in the evaluation of *Lactobacillus casei* were not subjected to statistical analysis, as there was no formation of inhibition halos.

Group P6 (zinc oxide and saline solution) did not show antimicrobial activity in the evaluation of *Enterococcus faecalis*, *Bacillus subtilis and Streptococcus mutans*. In this case, only the formation of a diffusion halo of the medicine itself

was observed. To obtain a more reliable confirmation of whether or not there would be microbial growth, a portion of the agar was collected from the inner zone of this halo, which was then introduced into liquid culture medium (BHI broth or specific medium) and taken for incubation in a bacteriological oven at 37°C/48h. After GSM, the material was analyzed under optical microscopy in order to visualize bacterial colonies.

For all microorganisms evaluated, group P3 showed greater antimicrobial activity compared to group P2. Only in the evaluation of *Streptococcus mutans* was no statistically significant difference observed between the means of these groups. Considering these results, it is worth highlighting that the absence of some component of the paste may influence the antimicrobial activity, as evidenced in this study. Chloramphenicol, for example, has a broad spectrum against bacteria such as Staphylococcus aureus and Escherichia coli and its mechanism of action occurs by inhibiting the bacterial protein synthesis process[22,23]. Tetracycline, in turn, is a broad-spectrum bacteriostatic agent that also inhibits the synthesis of bacterial proteins. On the other hand, tetracycline has disadvantages related to the potential for crown pigmentation and development of enamel hypoplasia in the successor premolar due to its high affinity for calcified tissues[15,24]. In addition, the inherent yellowish coloration and consequent darkening of the teeth, a fact that can be disadvantageous and discourage its use in aesthetic areas, such as upper incisors. To mitigate these disadvantages, the removal, replacement or reduction of the proportion of tetracycline may be an interesting and viable alternative. For this, additional in vitro and clinical studies are necessary to evaluate the antimicrobial properties and the efficacy of endodontic treatment with the use of a modified paste.

The results obtained showed that groups P1, P2, P3, P4 and P5 were effective against the six standard microorganisms, in all time intervals evaluated. In relation to the control pastes without antibiotics (P4, P5 and P6), group P6 was the only one to demonstrate ineffectiveness against the microorganisms tested, as the culture medium showed turbidity within 5 minutes, which led us to consider that this result could be related to the absence of eugenol in its composition.

Another result derived from this study refers to the fact that groups P4 and P5 presented means with very close values, statistically not significant, even with the discrepancy observed in the volume of eugenol between these groups (0.6ml in P4 and 0.0006ml in P5). Thus, the antimicrobial effectiveness observed in group P4 was attributed to the presence of eugenol in its composition, since zinc oxide evaluated separately (P6) showed no effectiveness on the standard microorganisms tested. Eugenol has a bactericidal action, due to its high affinity for the plasma membrane (lipid solubility) and its ability to inhibit the respiration and cell division of bacterial[25,26].

Ultimately, although this *in vitro* study presented interesting results, some methodological limitations should be mentioned. Among them, a considerable limitation refers to the fact that bacteria were not isolated, but rather in consortiums. Therefore, the findings

should be interpreted with caution, as we do not know whether the results of in vitro exposure to individual strains will be the same as the response of the necrotic canal system reaction. Furthermore, the response of each organism may vary and influence the dental prognosis. Overall, clinical studies can be performed based on our findings to confirm the clinical implications.

Conclusion

Based on the results of the present study, it can be concluded that the combination of all components of the CTZ paste (chloramphenicol, tetracycline and zinc oxide - 1:1:2 ratio, respectively) and eugenol as a vehicle for manipulation, presented antimicrobial activity against microorganisms *in vitro*. Nevertheless, when the components of the CTZ paste were evaluated separately, they presented smaller mean diameters of inhibition zones in relation to the combination of all drugs. Additionally, the individual use of eugenol presented antimicrobial efficacy, while the absence of this product tested in isolation did not present efficacy against microorganisms.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Correspondencia: Lucas Fernando Oliveira Tomáz Ferraresso, correo: lucas.ferraresso@unesp.br