

Evaluation of a Novel Endodontic Paste for Primary Teeth with Necrotic Pulp

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ABSTRACT

Background: The ideal root canal filling for endodontic treatment of primary teeth with necrotic pulp must have antimicrobial activity, not cause tooth discoloration or interfere with the eruption of permanent successor teeth. This study aimed to evaluate physicochemical properties of a novel paste (CZ Paste) for endodontic treatment of primary teeth with necrotic pulp and compare its antimicrobial activity to Chloramphenicol, Tetracycline, Zinc oxide (CTZ) and Zinc Oxide eugenol (ZOE) pastes. **Methods:** CZ, CTZ and ZOE pastes were homogenized with eugenol at the time of testing. Fourier Transform Infrared spectroscopy (FTIR) and Differential Scanning Calorimetry (DSC) tests were performed. Antimicrobial activity was assessed using agar diffusion test (ADT), direct contact test (DCT) and bactericidal effect assessment against *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa*. **Results:** FTIR showed that CZ paste has a similar profile to chloramphenicol spectrum and in DSC thermograms it demonstrated a slight change in endothermic transition temperature. In the ADT, CTZ paste and CZ paste presented

similar inhibition zone diameters and were higher than ZOE paste ($P < 0.05$). All pastes inhibited the growth of tested micro-organisms in DCT. Bactericidal assessment test showed that only CZ and CTZ pastes had bactericidal effect. **Conclusion:** CZ paste could be an alternative for endodontic treatment of primary teeth with necrotic pulp.

Key words: Primary tooth, Pulpectomy, Root canal filling materials, Antibiotic paste, Bacteria.

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INTRODUCTION

Conventional chemo-mechanical preparation of root canals in primary molars is advocated in cases of irreversible pulpitis or pulp necrosis in children.¹ However, as these procedures are laborious due to the complex anatomical characteristics of primary teeth,² dentists often choose to perform early extractions, which may trigger problems with occlusion and aesthetics.³ Among the root canal filling materials recommended, zinc oxide eugenol paste (ZOE paste) is highlighted, however, there is no consensus regarding the best paste to use in pulpectomies of primary teeth with necrotic pulp.⁴ The ideal endodontic material for primary teeth must have biocompatibility with periodical tissues, antimicrobial activity, not cause tooth discoloration or interfere with the eruption of permanent successor teeth.⁵ In order to increase the success rates of endodontic treatment in children, broad-spectrum antibiotics have been incorporated into these pastes to reduce and/or eliminate the micro biota and their insertion is limited to the pulp chamber, becoming technically more accessible to general practitioners.⁶⁻¹⁰ CTZ paste is a pharmacological dental material developed by Cappiello^{6,7} composed by chloramphenicol, tetracycline and zinc oxide. However, as pastes that contains tetracycline or its derivatives have the disadvantage of stain tooth structures the use of these substances has been discouraged.¹¹

Due to this reason, this study questioned whether modifying the original CTZ paste formulation would affect its properties in order to establish a novel paste to be used in dentistry. Therefore, the aim of this study was to evaluate physicochemical properties of a novel paste (CZ Paste) for

endodontic treatment of primary teeth with necrotic pulps and compare its antimicrobial activity to CTZ and ZOE pastes.

MATERIALS AND METHODS

Preparation of tested materials

CZ paste was composed of a ratio of one part of chloramphenicol and two parts of zinc oxide powders (Biodinamica, Ibiporã, Paraná, Brazil). CTZ paste powder was prepared in a specialised pharmacy (Galeno Pharmacy, Teresina, Piauí, Brazil) and individually encapsulated maintaining a ratio of one part of tetracycline, one part of chloramphenicol and two parts of zinc oxide.⁶ ZOE paste was composed of zinc oxide powder and eugenol (Biodinamica, Ibiporã, Paraná, Brazil).

An analytical balance (AG 200, Gehaka Electric, Electronic Industry and Commerce, São Paulo, Brazil) was used to weigh the components used they were prepared on sterile glass slabs at the time of testing. Powders were mixed following the power/liquid ratio of 1mg of powder to 4x10⁻⁴ ml eugenol (Biodynamics, Ibiporã, Paraná, Brazil), to ensure that all pastes had a similar consistence to toothpaste.

Physicochemical properties of CZ paste

Fourier transform infrared (FTIR) spectroscopy

CZ paste and its constituents (Chloramphenicol, Zinc Oxide and Eugenol) were analysed by FT-IR spectrometer (Perkin Elmer Spectrum 100 GTX) in range of 4000 cm⁻¹ and 400 cm⁻¹ at 4 cm⁻¹ resolution with

16 scans co-added and as KBr pellet. Collected spectra were investigated for possible chemical interactions between drugs and other components of formulation.

Differential scanning calorimetry (DSC)

A quantity of 5 mg of each sample (CZ paste and Chloramphenicol) was placed in an aluminium pan and all samples were heated at the rate of 10°C/min under nitrogen stream (50 mL/min) (DSC-60, Shimadzu®). The heating temperature was carried out in the range of 0 to 500°C. Zinc oxide could not be analysed because its melting point is too high and eugenol because it is a liquid at room temperature.

Antimicrobial activity

The antimicrobial activity of CZ paste was compared to CTZ and ZOE pastes using the agar diffusion test (ADT), direct contact test (DCT) and bactericidal effect assessment.

The bacterial strains of *Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 29212 and *Pseudomonas aeruginosa* ATCC 27853 (American-type culture collection, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil) were used.

Samples were cultured in Brucella broth supplemented with 2.5% yeast extract (Becton Dickinson and Company, Sparks, MD, USA) under aerobic conditions at 37°C for 24 hr. Then, the samples were inoculated in Brucella agar plates supplemented with 2.5% yeast extract (Becton Dickinson and Company, Sparks, MD, USA) and incubated. Afterwards, from isolated colonies, a suspension of each bacterial strain with a McFarland optical density of 0.5 (1.2×10^8 CFU/mL) was made into an 0.85% isotonic sodium chloride solution and used for all the experiments described below.

For the ADT test,¹² four 6 mm wells (one for each paste tested) were made on Mueller-Hinton agar plates (Becton Dickinson and Company, Sparks, MD, USA) and filled with the pastes. Then, 100 µL aliquots of each microbial species were inoculated into 8 mL tubes containing a semi-solid medium (Mueller-Hinton broth supplemented with 1% yeast extract and 0.8% Bacto agar, Becton Dickinson and Company, Sparks, MD, USA). These were homogenised and deposited over the plates with the previously filled wells. The seeded plates were maintained at room temperature for 1 hr and then incubated for 48 hr at 37°C in aerobic conditions. The experiments were carried out in triplicate.

Following the incubation period, the diameters of the inhibition zones were measured (in mm) using a calliper and the diameter mean was calculated. Data were analysed using the Graph Pad Prisma 5.0 software. Shapiro-Wilk test was applied to assess the normality of the variables. An analysis of variance (ANOVA) followed by Bonferroni's post-hoc test was used to assess the differences between the means of the groups ($P < 0.05$).

For DCT test,¹³ 96-well micro dilution plates were positioned vertically, the walls of the wells were coated with the same quantity of the three pastes tested (four wells for each paste). Then, 10 µL of each microbial suspension was inoculated into the pastes and the plates were then incubated for 1 hr under aerobic conditions at 37°C. Next, 245 µL of tryptone soya broth (TSB; Difco Laboratories, Detroit, MI, USA) were added to each well and the bacterial concentration was about 6×10^6 CFU/mL.

The culture medium (TSB) was used as the sterile control and the growth control (positive control) consisted of wells containing only the tested micro-organisms in TSB. A negative control containing only the pastes and broth was also performed. Absorbance readings were taken at the baseline and at 5, 10 and 24 hr at a wavelength of 630 nm (EL800 Universal Microplate Reader, Bio-Tek Instruments, Inc.). The plates were

vortexed for 2 min prior to each reading. Mean and standard deviation of the optical density (OD) values obtained for each sample were calculated. Following 24 hr of incubation of the micro plates used in DCT test, the bactericidal effect of the same pastes was also verified. 100 µL of suspension contained in each well was removed and inoculated into tryptone soy agar (TSA) (Becton Dickinson and Company, Sparks, MD, USA) plates, then incubated at 37°C for 48 hr under aerobic. This experiment was also carried out in quadruplicate. The colonies counting was performed. A bactericidal effect was considered when a reduction equal to or greater than 3 Log₁₀ CFU/mL in relation to the inoculum was found.¹⁴

RESULTS

Physiochemical properties of CZ paste

The FTIR spectra and DSC curves of CZ paste and its constituents are shown in Figure 1. Chloramphenicol had broad band at 3350 cm⁻¹ which was attributed to O-H bond stretching vibration, while at 3258 cm⁻¹, 1658 and 1554 can be also clearly identified with N-H, C=O stretching and N-H bending. The peaks at 1521 and 1352 cm⁻¹ are correlated with aromatic nitro compound. When several chlorine atoms are attached to one carbon atom, the band is usually more intense and the C-Cl vibration is observed at 778 cm⁻¹ (Table 1). Zinc Oxide infrared spectra shows signature bands at 500 and 442 cm⁻¹ attributed to Zn-O stretching.¹⁵ The spectra of Eugenol exhibit its signature peaks at 3515, 3072 and 3002 and 1638 cm⁻¹, corresponding to O-H stretching, Ar-H stretching and vinyl (C=C) stretching. In addition, sharp peaks at 1610 and 1512 cm⁻¹ were

Table 1: Frequency assignment of CZ paste components.

Compound	Wavenumber (cm-1)	Vibrational mode assignment and main contributions
Zinc Oxide	500 and 442 cm ⁻¹	Zn-O stretching
Chloramphenicol	3350 cm ⁻¹	O-H bond stretching vibration
	3263 cm ⁻¹	N-H stretching
	1686 cm ⁻¹	C=O stretching (amida I)
	1521/1350 cm ⁻¹	N-O stretching (NO ₂ - Ar)
	1065 cm ⁻¹	C-O-C stretching (primary alcohol)
	1018 cm ⁻¹	Ring bending
CZ Paste	845 cm ⁻¹	C-N Stretch (out of plan NH-bending)
	3350 cm ⁻¹	O-H bond stretching vibration
	3263 cm ⁻¹	N-H stretching
	1687 cm ⁻¹	C=O stretching (amida I)
	1518/1351 cm ⁻¹	N-O stretching (NO ₂ - Ar)
	1062 cm ⁻¹	C-O stretching (primary alcohol)
Eugenol	1018cm ⁻¹	Ring bending
	845 cm ⁻¹	C-N Stretching (out of plan NH-bending)
	3515 cm ⁻¹	O-H stretching
	3072 cm ⁻¹	Ar-H stretching
	3002/1638 cm ⁻¹	vinyl (C=C) stretching
	1610/1512 cm ⁻¹	C=C stretching of the aromatic moiety
	1265 cm ⁻¹	C-O-C stretching

also found in eugenol, which can be due C=C stretching of the aromatic moiety and at 1265 cm^{-1} , which correspond to C-O stretching. CZ paste presented a similar profile with the chloramphenicol spectrum with weaker absorbance because of the presence of other compounds.

DSC curve of Chloramphenicol has shown a sharp endotherm band at 152.63°C attributed to de melting point (T_m) and in CZ curve the T_m of Chloramphenicol has been seen at 151.4°C .

Antimicrobial activity

The mean diameters of the microbial growth inhibition zones for the pastes in the ADT are presented in Table 2. In general, the CZ paste and CTZ pastes presented similar inhibition zone diameters and were higher than the ZOE paste ($P < 0.05$).

CZ, CTZ and ZOE pastes inhibited the growth of all the tested micro-organisms when compared to positive control (Figure 2A, B, C and D, respectively).

The bactericidal assessment test showed that the CZ paste and CTZ had positive bactericidal effect against all tested micro-organisms, while ZOE paste showed no effect.

DISCUSSION

CZ paste was developed as an alternative material for pulpectomies in primary teeth from a modification of CTZ paste by removing tetracycline of its formula. It also should be inserted just at the entrance of root canals and perhaps could be used in both anterior and posterior teeth, without the disadvantage of staining tooth structure promoted by tetracycline in the first formulation. However, further studies are necessary to establish this formulation and randomised clinical studies must be carried out in order to compare CZ paste with other techniques recommended in the literature.

In order to describe physiochemical properties of CZ paste, FTIR and DSC tests were performed. FTIR test describe several interactions between the drug and active molecules to analyse the spectra of each component of the formula.¹⁶ These substances presented several absorption peaks as described. The paste CZ presented similar profile to the chloramphenicol spectrum with weaker absorbance because of the other components of the paste, moreover, shifting also was not observed indicating no significant interactions between Zinc Oxide, Chloramphenicol and Eugenol. Sterren *et al.*¹⁷ reported possible molecular interactions and changes in solid-state forms into Chloramphenicol and different Amino Acids.

The bands of the solid system combination resulted in no interactions between the pure active ingredients while the antimicrobial activity of the antibiotic was preserved. DSC thermograms of Chloramphenicol and CZ paste demonstrated that the incorporation of particles to use as a paste changed a little the endothermic transition temperature. The displacement may have been caused due to the presence of the other paste components. The thermal decomposition of Chloramphenicol carried out at 258°C . The DSC curve of CZ did not show a band of zinc oxide because its thermal decomposition occurs over 600°C . However, the CZ curve has shown bands at 258°C and 313°C that correspond the thermal decomposition of chloramphenicol and eugenol. This could indicate that there were no interactions between the compounds, corroborating the FTIR results, corroborating to a previous study that showed that the combination of Chloramphenicol/Amino Acids had the melting endotherm temperature preserved and there was no interaction between them.¹⁷ CTZ paste contains zinc oxide eugenol and two broad-spectrum antibiotics: tetracycline and chloramphenicol. It presents an effective antimicrobial activity,¹⁸ biocompatibility¹⁹ and satisfactory clinical and radiographic results.^{9,10} Besides this, the blood cellularity interface in the tooth socket of primary molars treated with CTZ paste is similar to those of healthy, exfoliated teeth and physiologically different from untreated decayed teeth.²⁰ Primary teeth with necrotic pulps present polymicrobial infections. There is a predominance for anaerobic (facultative and moderate) species, gram-positive cocci and gram-negative coccobacilli.²¹ The micro-organisms used were referenced in other studies and are representative of the microbiota of primary molars with necrotic pulps.¹⁸ CZ paste and CTZ paste presented a similar antimicrobial activity, which

Table 2: Inhibition zone diameters (in mm) provided by ADT.

Tested bacteria	CZ Paste	CTZ Paste	ZOE Paste
	Mean (S.D.)	Mean (S.D.)	Mean (S.D.)
<i>E. faecalis</i>	36.0 (3.5) ^c	34.3 (4.0) ^c	24.0 (2.0) ^{ab}
<i>S. aureus</i>	33.0 (1.7) ^c	30.0 (0.0) ^c	11.3 (1.2) ^{ab}
<i>P. aeruginosa</i>	32.0 (0.0) ^c	30.3 (2.3) ^c	11.3 (0.6) ^{ab}

S.D. stands for Standard deviation.

Inter-pastes significant difference following an ANOVA and Bonferroni's post-hoc test ($P < 0.05$). **a** – difference from the CZ Paste, **b** – from the CTZ paste and **c** – from the ZOE paste.

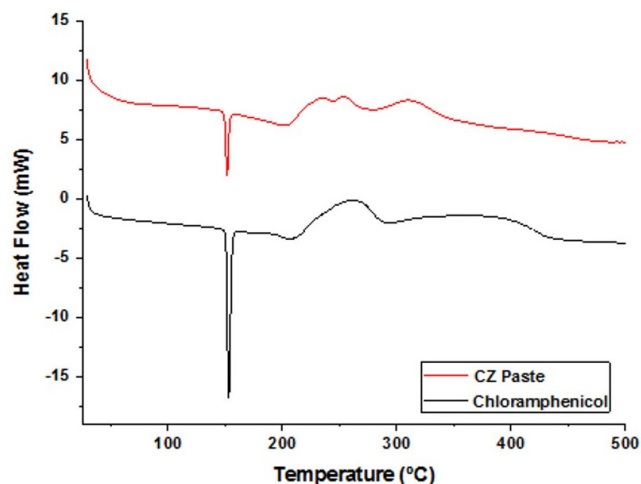


Figure 1: FTIR Spectra of CZ paste, Chloramphenicol, Zinc oxide and Eugenol and DSC curves of CZ paste, Chloramphenicol.

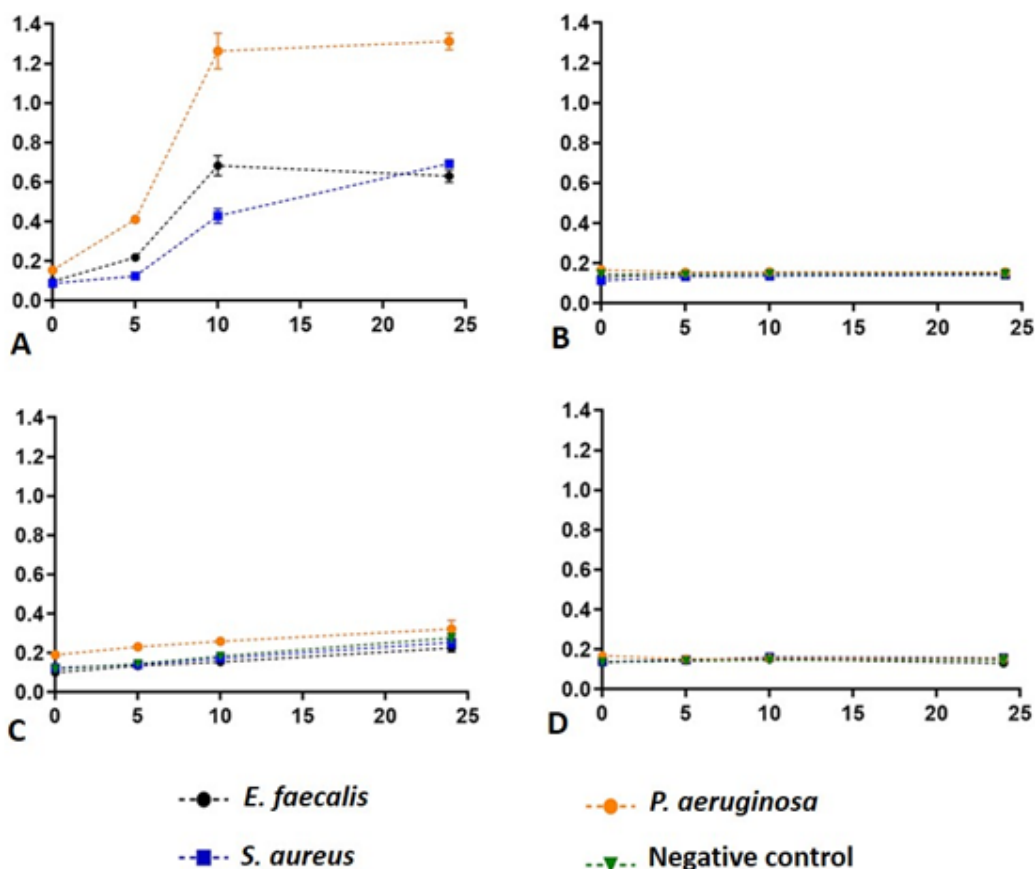


Figure 2: Antimicrobial activity of the pastes in the DCT according to time (h). Each point in the growth curve expresses the mean \pm SEM optical density (OD) values obtained for four wells. A – Bacterial growth (positive control), B – CZ paste test, C – CTZ paste test and D – ZOE paste test.

was higher than ZOE paste in both agar diffusion and direct contact tests. This corroborates with Amorim *et al.*¹⁸ which used the same method and observed that CTZ paste had a more effective antimicrobial activity than ZOE paste for all the micro-organisms. Tetracycline and chloramphenicol are bacteriostatic antibiotics that target the protein synthesis of Gram-positive and Gram-negative bacteria and have limited synergy.^{22,23} The absence of synergistic activity of that combination could explain why the CTZ paste did not present significantly better results than the CZ paste.

On the direct contact test ZOE paste showed a bacteriostatic effect; however, it presented no bactericidal effect on the growth of the micro-organisms afterwards, which differs from the study by Heyder *et al.*¹³ which found that zinc oxide eugenol cement led to a significant reduction in the colony-forming units of *E. faecalis*, *F. nucleatum* and *P. gingivalis*. This may have occurred due to the fact that ZOE paste loses its antimicrobial action once it sets.²⁴ One of the goals of paediatric dentistry is to perform endodontic treatment in primary teeth to maintain its function during the biological cycle.^{1,4} Traditional pulpectomy procedure in primary molars with necrotic pulp requires instrumentation of root canal system, disinfection with antimicrobial irrigants solutions and filling with absorbable and antibacterial materials.^{1,4,5} ZOE paste is a traditional and largely recommended material for conventional treatments involving root canal instrumentation of the primary teeth.^{1,4} Its antibacterial effect is mainly attributed to the action of eugenol that causes protein denaturation.^{5,10,24} It is assumed that in view of the anatomical complexity of primary molars, its action is not

sufficient enough to reduce the bacterial contamination and the use of antimicrobial irrigating substances is mandatory when instrumentation is performed.²⁵

CONCLUSION

CZ paste could be an alternative for endodontic treatment of primary teeth with necrotic pulp.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

mm: milimetre; **cm:** centimetre; **μ L:** microlitre; **mL:** millilitre; **mg:** milligram; **min:** minute; **$^{\circ}$ C:** degree celsius; **hr:** hour; **CFU:** colony-forming unity.

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