

UNIVERSIDADE DE SÃO PAULO  
FACULDADE DE ODONTOLOGIA DE BAURU

PEDRO HENRIQUE SOUZA CALEFI

**Analysis of pH and antimicrobial activity of Ambroxol  
Hydrochloride and N-Acetylcysteine pastes to be used as root canal  
medication**

**Análise do pH e atividade antimicrobiana das pastas de Cloridrato  
de Ambroxol e N-Acetilcisteína utilizadas como medicação  
intra canal**

BAURU  
2020



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Dissertação constituída por artigo apresentada à Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Mestre em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Endodontia.

Orientador: Prof. Dr. Marco Antonio Hungaro Duarte

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# FOLHA DE APROVAÇÃO



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## DEDICATÓRIA

Dedico este trabalho aos meus pais ***José Benedito Calefi*** e ***Denise da Silva Souza Calefi*** e à minhas irmãs ***Mariana Souza Calefi*** e ***Ana Elisa Souza Calefi***.

Com todo meu carinho e respeito, amo vocês.

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# **ABSTRACT**

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## ABSTRACT

The aim of the present study was to evaluate the pH and antimicrobial activity of N-Acetylcysteine (NAC) and Ambroxol Hydrochloride (AMB) pastes comparing with calcium hydroxide paste (CHP). Experimental pastes made of NAC, AMB and CHP, employing propylene glycol as vehicle with 500 mg / 0,5 mL concentration, were prepared for testing. The pH was measured after 3 hours, 3 and 7 days with a calibrated pHmeter; freshly pastes were inserted into tubes and immersed into flasks containing 10 mL of ultrapure water; the pH of ultrapure water was used as control. For in vitro antimicrobial activity evaluation, biofilm of *Enterococcus faecalis* was induced on 60 blocks of bovine dentin which were divided in 3 experimental and a control (no treatment) groups (n=15). The pastes were prepared and after 7 days of contact the blocks were analyzed under a confocal microscope using the Live/Dead dye; the percentage of live cells was measured using biolmage software. The NAC and AMB presented acidic pH and the CHP presented pH higher than 11 in all periods. There were statistically significant differences between all groups at all periods analyzed ( $P<0.05$ ). The NAC and AMB groups offered a lower number of viable cells than the CHP group, which presented no significant differences in comparison to the CON group. The AMB presented greater antimicrobial action than CHP ( $P<0.05$ ). We can conclude that the N-Acetylcysteine and Ambroxol Hydrochloride pastes presented acid pHs which, against *E. faecalis* biofilm, presented antimicrobial activity, with Ambroxol Hydrochloride being more effective. Further studies should be done in order to suggest its use on the endodontic therapy.

**Keywords:** *Enterococcus faecalis*; Biofilm; Calcium hydroxide; N-Acetylcysteine; Ambroxol hydrochloride; Antimicrobial action.

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# RESUMO

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## RESUMO

O objetivo do presente estudo foi avaliar o pH e a atividade antimicrobiana das pastas N-acetilcisteína (NAC) e Cloridrato de Ambroxol (AMB) em comparação à pasta de Hidróxido de Cálcio (CHP). Pastas experimentais feitas de NAC, AMB e CHP, empregando propilenoglicol como veículo com concentração de 500 mg / 0,5 mL, foram preparadas para teste. O pH foi medido após 3, 72 e 168 horas com um medidor de pH calibrado; pastas frescas foram inseridas em tubos e imersas em frascos contendo 10 mL de água ultrapura; o pH da água ultrapura foi utilizado como controle. Para avaliação da atividade antimicrobiana, o biofilme de *Enterococcus faecalis* foi induzido *in vitro* em 60 blocos de dentina bovina, os quais foram divididos em três grupos experimentais e um controle (sem tratamento) (n = 15). As pastas foram preparadas e após 7 dias de contato os blocos foram analisados sob microscopia confocal utilizando o corante Live / Dead; a porcentagem de células vivas foi mensurada usando o software bioImage. O NAC e AMB apresentaram pH ácido e o CHP apresentou pH superior a 11 em todos os períodos. Houve diferenças estatisticamente significantes entre todos os grupos em todos os períodos analisados (P <0,05). Os grupos NAC e AMB ofereceram um número menor de células viáveis que o grupo CHP, que não apresentou diferença significativa em comparação ao grupo CON. O AMB apresentou maior ação antimicrobiana que o CHP (P <0,05). Concluiu-se que as pastas de N-Acetilcisteína e Cloridrato de Ambroxol apresentaram pH ácido que, contra o biofilme de *E. faecalis*, apresentou atividade antimicrobiana, sendo o Cloridrato de Ambroxol mais eficaz. Outras análises devem ser feitas com ambas as substâncias a fim de sugerir seu uso na terapia endodôntica.

**Palavras-chave:** *Enterococcus faecalis*; Biofilme; Hidróxido de Cálcio; N-Acetilcisteína; Cloridrato de Ambroxol; Atividade antimicrobiana

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# **1 INTRODUCTION**

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## **1 INTRODUCTION**

The endodontic infection is a true challenge for clinicians, even after the biomechanical preparation, due to the number of bacteria inside the root canal system and the anatomical complexity of each case (Vera *et. al* 2012).

The use of intracanal medication between sessions before the obturation, optimizes the success rate of the endodontic therapy, especially the Calcium Hydroxide paste in cases of endodontic failure (Zancan *et. al* 2016; 2019). However, *E. faecalis*, one of the most frequent bacteria in those cases, some strains of this bacteria have shown resistance to the alkaline action of the Calcium Hydroxide (Evans *et. al* 2002; Nakajo *et. al* 2006; Chávez de Paz *et. al* 2007).

Considering the importance of the antiseptics of the root canal system, and aiming a direct action on the biofilm, the mucolytic agent N-Acetylcysteine has been showing favorable results in this scenario (Choi *et. al* 2018; Costa *et. al* 2017). Moreover, this composite has shown in previous studies, some antimicrobial action against *E. faecalis* (Ulusoy *et. al* 2016; Quah *et. al* 2012).

Another substance used to help to disaggregate and solve mucus, is the Ambroxol Hydrochloride, often used in pulmonary infections (Cataldi *et. al* 2014). This one acts in the composition of mucus, lowering its viscosity and thickness, what could be favorable to pass through the polysaccharides of the biofilm and reach the bacterial cells (Li *et. al* 2017; Hull *et. al* 2018).

Considering the resistance of the *E. faecalis* to the Calcium Hydroxide, one of the most used intracanal medication in endodontic therapy, the aim of the present study was to evaluate the pH and antimicrobial activity of the N-Acetylcysteine and Ambroxol Hydrochloride based pastes, comparing with Calcium Hydroxide paste, against an *E. faecalis* biofilm.

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# **4 CONCLUSIONS**

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## **4 CONCLUSIONS**

The N-Acetylcysteine and Ambroxol Hydrochloride pastes presented acid pHs which, against *E. faecalis* biofilm, presented antimicrobial activity, with Ambroxol Hydrochloride being more effective. The use of both substances should be further studied in order to suggest its use on the endodontic therapy.



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**ANNEX**

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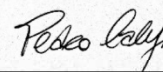


**DECLARAÇÃO DE USO EXCLUSIVO DE ARTIGO EM DISSERTAÇÃO**

Declaramos estar cientes de que o trabalho **Analysis of pH and antimicrobial activity of Ambroxol Hydrochloride and N-Acetylcysteine pastes to be used as root canal medication** será apresentado na dissertação do aluno Pedro Henrique Souza Calefi e que não foi e nem será utilizado em outra dissertação/tese dos Programas de Pós-graduação da FOB-USP.

Bauru, 03 de Abril de 2020.

Pedro Henrique Souza Calefi



Assinatura

Rafaela Fernandes Zancan



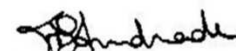
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