

Analysis of pH and release of calcium of association between melaleuca alternifolia oil and calcium hydroxide

Análise de pH e liberação de cálcio da associação entre óleo de melaleuca alternifolia e hidróxido de cálcio

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Resumo

Introdução: O uso de medicações intracanais com propriedades antimicrobianas é fundamental para descontaminação dos canais radiculares durante o tratamento endodôntico. O hidróxido de cálcio é utilizado como medicação intracanal por apresentar excelentes propriedades. O óleo de *Melaleuca alternifolia* apresenta importância medicinal demonstrando ação antifúngica e bactericida comprovada contra patógenos humanos. **Objetivo:** Avaliar aspectos físico-químicos da associação do óleo de *Melaleuca Alternifolia* com hidróxido de cálcio, como: pH e liberação de cálcio durante diferentes períodos. **Material e método:** O pó do hidróxido de cálcio foi adicionado aos veículos até a concentração de 72mg/0,1mL. Foram divididos três grupos: Grupo I: Hidróxido de Cálcio + Água Destilada; Grupo II: Hidróxido de Cálcio + Propilenoglicol; Grupo III: Hidróxido de Cálcio + Óleo de *Melaleuca*. O pH de cada grupo foi medido após 10 minutos, 24, 48 horas, 7, 15 e 30 dias após a espatulação por um pHmetro. A liberação de cálcio foi analisada através da espectrometria de absorção atômica equipada com uma lâmpada cátodo para cálcio. Os dados foram analisados estatisticamente pelos testes de Kruskall-Wallis e Dunn. **Resultado:** O grupo II apresentou pH elevado, semelhante ao grupo III, permanecendo uniforme aos 15 e 30 dias. A liberação de cálcio iniciou em 24 horas, de forma semelhante nos grupos II e III e seu pico de liberação deu-se em 48 horas. **Conclusão:** A associação do Óleo de *Melaleuca* com hidróxido de cálcio apresentou bons resultados quanto à análise de pH e liberação de cálcio, demonstrando ação semelhante ao propilenoglicol + hidróxido de cálcio.

Descritores: Endodontia; hidróxido de cálcio; óleo de *melaleuca alternifolia*.

Abstract

Introduction: The use of intracanal medications with antimicrobial properties is essential for decontaminating root canals during endodontic treatment. Calcium hydroxide is used for this because of its excellent properties. *Melaleuca alternifolia* oil has shown medicinal importance by demonstrating antifungal and bactericidal action against proven human pathogens. **Objective:** To evaluate the physical and chemical aspects such as pH and calcium release, of *Melaleuca alternifolia* oil associated with calcium hydroxide, during different time intervals. **Material and method:** Calcium hydroxide powder was added to vehicles to reach a concentration of 72mg / 0.1mL. Three groups were formed: Group I: Calcium Hydroxide + Distilled Water; Group II: Calcium hydroxide + Propylene Glycol; Group III: Calcium hydroxide + *Melaleuca* oil. The pH of each group was measured after time intervals of 10 minutes; 24 and 48 hours; 7, 15 and 30 days after tooling by a pH meter. Calcium release was analyzed by atomic absorption spectrometry equipped with a calcium hollow cathode lamp. Data were statistically analyzed by using the Kruskall-Wallis and Dunn test. **Result:** Group II showed high pH, similar to group III that remained uniform at 15 and 30 days. Calcium release that began after 24 hours, was similar in Groups II and III, and showed a peak release in 48 hours. **Conclusion:** The association of *Melaleuca* oil with calcium hydroxide showed good results in the pH and calcium release analyses, and showed action similar to that of propylene glycol + calcium hydroxide.

Descriptors: Endodontics; calcium hydroxide; *melaleuca alternifolia* oil.

INTRODUCTION

The use of intracanal medication that has antimicrobial properties is of fundamental importance for decontaminating root canals during the stages of endodontic treatment. Calcium

hydroxide is widely used for this purpose, because it has excellent biologic and antimicrobial properties. This compound, used as therapeutic intracanal agent has demonstrated antimicrobial effects

in root canals because of its effective bactericide and bacteriostatic action. When used as a delayed dressing, its action has broad scope, because it has a destructive effect on the bacterial cell membrane and protein structure¹, considering that a set of microorganisms are responsible for infections that occur within the root canal².

In cases of colonization of the dentinal tubules and ramifications of the main canal, frequent findings are microorganisms of species such as *Candida albicans* and gram-positive anaerobic bacteria *Enterococcus faecalis*, and in cases where secondary infections and the evolution of peri-radicular lesions are concerned³⁻⁵. However, according to Hammer et al.¹, calcium hydroxide appears to be incapable of disinfecting dentinal tubules contaminated by *E. Faecalis*; and yeasts such as *Candida albicans*, according to Lopes, Siqueira⁶. Therefore, options need to be studied to potentiate its action in decontaminating the root canal system^{7,8}.

According to Estrela, Figueiredo⁹, calcium hydroxide has an elevated pH that causes damaging biologic effects on bacterial cells. The effect of this high pH of calcium hydroxide (12.6) influenced by hydroxyl ion release, is capable of changing the integrity of the cytoplasmic membrane by means of chemical injuries to the cellular organic components and nutrient transport, or by means of destroying phospholipids and fatty acids of the cell plasma membrane, observed by the lipid peroxidation process - a saponification reaction - also known as lipid hydrolysis.

This also provides calcium hydroxide with alkalinity, the capacity to induce mineralized tissue formation, stimulate enzymes such as alkaline phosphatase, and inhibit acid phosphatase of osteoclastic origin. Moreover, it has been pointed out that the calcium ion, part of the immune reaction, may also be important in pulp and periodontal repair, because it activates calcium-dependent adenosine triphosphatase, associated with mineralized tissue formation¹⁰.

Melaleuca (Tea-Tree) oil has been shown to be of great medicinal importance because it has proven antifungal and bactericidal action against various human pathogens. In a study, Oliva et al.⁸ demonstrated its antifungal activity, and so did Siqueira et al.¹¹. According to Savage et al.¹², a large portion of the bacteria present in root canals are sensitive to *Melaleuca* oil, including *E faecalis* and *P. Aeruginosa*, although the oil needs to be at a higher concentration to inhibit them. Papadopoulos et al.¹³, also demonstrated equivalent activity against *P. Aeruginosa*, and for this reason, it began to be used in Dentistry.

Tea-Tree Oil (TTO), is an essential oil extracted from the plant *melaleuca alternifolia* (popularly also known as the tea tree) that is mainly developed in plantation areas (with higher prevalence in Australia), according to Rôças et al.¹⁴. *Melaleuca* oil, at present used as an antimicrobial and preventive agent, also presents antiviral, antifungal and anti-inflammatory action. It has demonstrated significant phytotherapeutic action in the treatment of problems related to endodontic infections and other oral problems such as caries and periodontal disease¹⁵.

Considering the need to complement the antimicrobial action of calcium hydroxide in cases of bacterial resistance, and considering the antimicrobial potential of tea-tree oil, it is necessary to investigate a possible joint action by associating the two agents, with a view to seeking a synergism and increasing the antimicrobial power for use against endodontic infections.

The aim of the present study was to evaluate the physical-chemical aspects of associating Tea-Tree oil with calcium hydroxide for use as intra-canal medication, such as: pH and calcium release over the course of different time intervals.

MATERIAL AND METHOD

Firstly, the calcium hydroxide powder (Biodinâmica - Ibioporã, Paraná, Brazil) was added to 0.1mL of vehicle until it reached a concentration of 72mg/0.1mL. Thus, the groups were divided according to the vehicles: Group I: Calcium Hydroxide + Distilled Water (Laboratório de Química da Unicesumar – Maringá, Paraná, Brazil); Group II: Calcium Hydroxide + Propylenoglycol (Médformula Farmácia de Manipulação - Maringá, Paraná, Brazil); Group III: Calcium Hydroxide + *Melaleuca* Oil (Médformula Farmácia de Manipulação - Maringá, Paraná, Brazil).

According to the Certificate of Analysis of the *Melaleuca* oil, it was extracted from the leaves and stems of the plant *Melaleuca Alternifolia*, with a relative density of 0.894 g/mL, refraction of 1.475, specific rotation of +8° and purity GC 40.3%, and was of Chinese origin.

Three samples of each group were prepared to perform the pH readout. After preparing the pastes, they were placed in receptacles measuring 0.3 mm in diameter and 10 mm long that were hermetically sealed at one extremity. Subsequently, the samples were stored in beakers containing a quantity of 10 ml distilled water. The pH of each group was measured after time intervals of 10 minutes, 24, 48 hours, 7, 15 and 30 days, after having been spatulated by means of a pH-meter (BEL Engineering – model: w3b pH-meter – Monza, Milan, Italy) calibrated with solutions with pH 4.0 and 7.0 before each time of use. The measurements were repeated three times and a mean of the values was calculated.

For calcium ion release analysis, the samples were prepared in the same way as described for the pH analysis. Fifty-four samples were made to carry out this verification, according to the different groups. Calcium ion release was analyzed by means of flame photometry (Analyser Instrumentação Analítica – Penha, São Paulo, São Paulo) with the use of atomic absorption spectrometer equipped with a calcium hollow cathode lamp, under the following conditions: current 3 mA; nitrous oxide gas, oxidation-reduction stoichiometry, wavelength of 422.7 nm, and gap amplitude of 0.2 nm. A standard solution of 100 mg/L of calcium was prepared and diluted in HCl at 0.1 mol/L with the purpose of constructing a calibration curve. When necessary, the samples were diluted in a solution of HCl at 0.1 mol/L, with the same acid solution being used as the blank.

The data were statistically analyzed by the Kruskal-Wallis test, followed by the Dunn Test, at a level of significance of 5%.

RESULT

Table 1 presents the pH analysis of the groups. The authors noted that in the first 10 minutes, Group I (calcium hydroxide + distilled water) presented a pH similar to that of Group III (calcium hydroxide + *melaleuca* oil). However, Group II (calcium hydroxide + propylenoglycol) presented a higher pH, that was

statistically significant ($p < 0.05$), when compared with Groups I and III, indicating a high initial pH.

In the time interval of 24h, Group I maintained a stable pH, presenting no significant elevation peaks. However, Group II maintained the high pH, and so did Group III. After 48 hours, both Groups presented a similar pH, remaining in continuous progress over the course of the 15 and 30 days of analysis, as demonstrated in Figure 1.

Table 2 presents the calcium release analysis. The authors verified that there was practically no calcium release after the first 10 minutes, but as from 24 hours there was, particularly in Groups II and III.

After 24 hours, Group I continued to show low calcium ion release. However, its peak release occurred after 48 hours, in a manner similar to that of Groups I, II and III. Close to 15 days, the only group that still presented significant calcium release was Group II, while in Groups I and III release of this ion fell in a similar manner, as demonstrated in Figure 2.

However, close to the 30 days of analysis, there was another peak of calcium ion release in a uniform manner, in the three different groups, which allowed the authors to affirm that calcium continued to be released one month after spatulation of the pastes.

DISCUSSION

The purpose of the vehicle used in preparing the calcium hydroxide pastes was to promote the ionic dissociation of calcium and hydroxyl ions. These substances may influence the H of the paste; the speed of ion diffusion through the dentinal tubules; and the buffer capacity of dentin. According to the vehicle used, the medication may present different viscosities, so that a solution flows easily when it has low viscosity. Therefore, the type of vehicle used may facilitate ionic dispersion from the paste¹⁶.

During the first time intervals of pH analysis, the authors could verify that Group I presented relatively low pH when compared with Group II. This could have been related to the fact that the vehicle used in Group I was shown to be more soluble, thus facilitating the occurrence of evaporation from the paste.

At 15 and 30 days, all the pastes analyzed showed a pH value below 8.5. Therefore, the pH of all the calcium hydroxide pastes fell according to time, as occurred in the study of Viana et al.¹⁶. Thus,

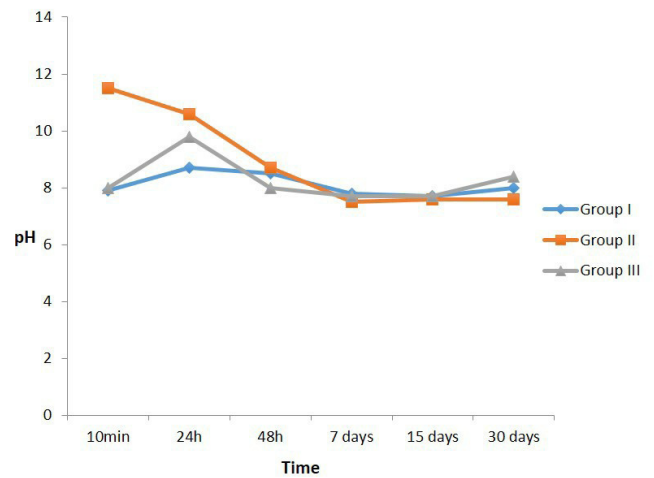


Figure 1. Variation in pH according to time of Group I, Group II and Group III.

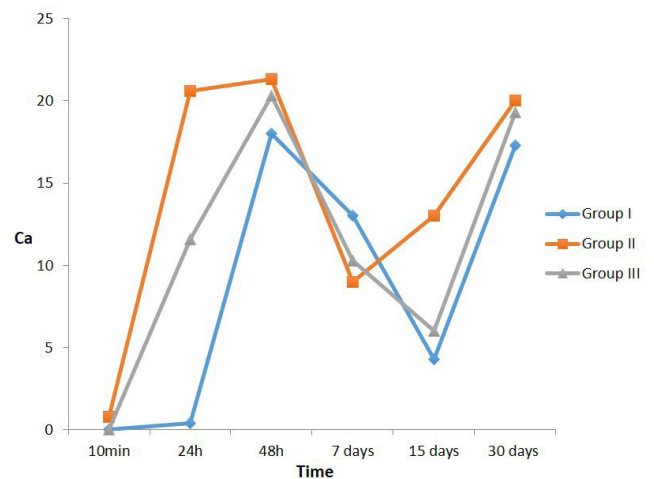


Figure 2. Variation in calcium release according to time of Group I, Group II and Group III.

Table 1. Mean pH values of Group I, Group II and Group III according to the different time intervals

Groups	10 minutes	24 Hours	48 Hours	7 Days	15 Days	30 Days
I	7.9 ^a	8.7	8.5	7.8	7.7	8.0
II	11.5 ^b	10.6	8.7	7.5	7.6	7.6
III	8 ^a	9.8	8	7.7	7.7	8.4

Different letters indicate statistically significant differences in the columns ($p < 0.05$).

Table 2. Mean calcium release values, in milligrams of Group I, Group II and Group III, according to the different time intervals

Groups	10 minutes	24 Hours	48 Hours	7 Days	15 Days	30 Days
I	0	0.4 ^a	18.6	13	4.3 ^a	17.3 ^b
II	0.8	20.6 ^b	21.3	9	13.6 ^b	20 ^a
III	0	11.6 ^b	20.3	10.3	6 ^a	19.3 ^{ab}

Different letters indicate statistically significant differences in the columns ($p < 0.05$).

the pastes prepared with both oily and aqueous vehicles conserved similar mean pH values during the final time intervals analyzed.

As the pH of calcium hydroxide is approximately 12.5, diverse species of bacteria found in infected root canals are eliminated after a short period of time in direct contact with this alkaline substance. This compound is capable of providing an environment that prevents the evolution of infections and establishes a direct effect on the bacterial cells that prefer to develop in an acid environment. Therefore, the alkaline environment triggered by calcium hydroxide impedes the development of infection, and its effect is directly proportional to its alkaline potential. This is because high degrees of pH produce bacterial destruction, thereby favoring endodontic treatment¹⁷.

Thus, pastes that have a high pH have a significant influence on combating endodontic infections present within the root canals, in addition to helping with the chemical-mechanical preparation performed during endodontic procedures.

Regarding calcium release during the initial time intervals of analysis, the authors perceived that Group I presented low levels of ion release when compared with Groups II and III. There was relative stabilization of the condition as from 48 hours, particularly in Group I that showed diminished ion release. This may be justified due to the higher level of evaporation from the paste prepared with distilled water because the vehicle was more aqueous, and therefore more volatile. Ferreira et al.¹⁸ apud Vivan et al.¹⁹ studied pH and calcium release levels of three calcium hydroxide-based products, by using camphorated paramonochlorophenol [PMCC] as vehicle, saline paste and LC paste, and concluded that both PMCC and saline paste were inhibitors of increase in pH and calcium release.

Montero, Mori²⁰, conducted a study to evaluate the association between calcium hydroxide + propolis and concluded that propolis could be used as a vehicle for calcium hydroxide in intracanal

medications. Although propolis is an oily vehicle, its capacity for diffusion when in a paste together with calcium hydroxide was similar to the capacity of the action of propylenoglycol. Moreover, the components of the propolis solution did not harm the dissociation of calcium hydroxide, and also diffused through the dentinal tubules²⁰.

In the present study, the authors were able to evaluate the effectiveness of *Melaleuca* oil, thus demonstrating that oily vehicles may be efficient in endodontic treatment during the delayed dressing stage, as was also found in the study of Montero, Mori²⁰.

Although the capacity for action of the paste containing *Melaleuca* oil + calcium hydroxide was similar to that of propylenoglycol, the oil may be an indication of better quality, due to the fact that it could contribute with its antimicrobial action. Further studies are necessary to question the biocompatibility of this paste, and confirm the use of *Melaleuca* oil as a vehicle for calcium hydroxide.

The use of Tea-Tree oil in Dentistry must be better investigated, so that it may be considered an option in the future, because of its potential it may provide important benefits and make a significant contribution to Endodontics by favoring the combat against endodontic infections present within root canals, allowing its use as intracanal medication for up to 30 days.

CONCLUSION

Melaleuca oil presented an action similar to that of propylenoglycol, which is the reference vehicle in association with calcium hydroxide in intracanal medications. It showed good results in the pH and calcium release analyses, demonstrating that it did not impede the release of hydroxyl from the calcium ions, thereby allowing the calcium hydroxide to perform its function.

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CONFLICTS OF INTERESTS

The authors declare no conflicts of interest.

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