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MORPHOLOGIC AND MORPHOMETRIC ANALYSIS OF THE ROOT CANAL APICAL THIRD CLEANING AFTER BIOMECHANICAL PREPARATION USING 3.3% *RICINUS COMMUNIS* DETERGENT AND 1% NaOCL AS IRRIGATING SOLUTIONS

*ANÁLISE MORFOLÓGICA E MORFOMÉTRICA DA LIMPEZA DE CANAIS RADICULARES APÓS PREPARO BIOMECÂNICO UTILIZANDO DETERGENTE DERIVADO DO ÓLEO DA MAMONA (*RICINUS COMMUNIS*) A 3.3% E NaOCL A 1%COMO SOLUÇÕES IRRIGANTES*

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ABSTRACT

This study evaluated, by morphologic and morphometric analyses, the cleaning of apical third of root canals instrumented with nickel-titanium rotary files using different irrigating solutions. Twenty-seven single-rooted mandibular premolars were assigned to three groups (n=9), according to the irrigating solution used: Group I, distilled and deionized water; Group II, 1% NaOCl; and Group III, 3.3% *Ricinus communis* detergent. Biomechanical preparation was performed with Protaper Plusâ nickel-titanium files as follows: S1, SX and S2 at the cervical and middle thirds, and 25/02, 25/04, 25/06, 30/02, 30/04 and 30/06 to complete the instrumentation, operating at 1 mm from the root apex. Irrigation was done at each file change with 2 mL of irrigating solution, totalizing 20 mL for each tooth. After biomechanical preparation, the apical thirds were serially sectioned and histologically processed. The cross-sections were examined by an optical microscope (X40) connected to a computer. The images were captured and analyzed using a computer software and submitted to morphometric analysis with aid of a grid. The percentage of debris remaining at the apical third was calculated. Data were submitted to statistical analysis by ANOVA and Tukey test. The results showed no statistically significant difference ($p>0.01$) between the groups irrigated with 1% NaOCl and 3.3% *Ricinus communis* detergent, which presented the lowest percentage of debris at the apical third, 8.49% and 10.11%, respectively. The group irrigated with distilled and deionized water had the highest percentage of debris (15.58%) and was statistically different from the other groups ($p<0.01$). It may be concluded that 3.3% *Ricinus communis* detergent and 1% NaOCl had similar cleaning effectiveness on removal of debris from root canals.
Uniterms: Root canal cleaning; Rotary instrumentation; Irrigating solution; *Ricinus communis.*

RESUMO

Estudou-se, por meio de análise morfológica e morfométrica, a capacidade de limpeza promovida pela instrumentação rotatória com limas Ni-Ti e irrigação com diferentes soluções. Vinte e sete pré-molares inferiores foram distribuídos em três grupos (n=9), de acordo com a solução irrigante utilizada: Grupo I, água destilada e deionizada; Grupo II, NaOCl a 1% e Grupo III, detergente derivado do óleo de mamona (*Ricinus communis*) a 3,3%. O preparo biomecânico foi realizado com sistema rotatório de Ni-Ti Protaper Plus, obedecendo a seguinte ordem de uso dos instrumentos: S1, SX e S2 para o terço cervical e médio, seguida do 25/02, 25/04, 25/06, 30/02, 30/04 e 35/02, completando a instrumentação todos operando 1 mm aquém do ápice. A irrigação foi realizada a cada troca de instrumento com 2 mL de solução irrigante, totalizando um volume de 20 mL para cada dente. Após o preparo biomecânico, os terços apicais dos dentes foram submetidos ao processamento histológico. Os espécimes foram analisados em microscópio óptico (40X) conectado a um computador. As imagens foram capturadas e analisadas utilizando-se softwares específicos e submetidas à análise morfométrica por meio de uma grade de integração. A porcentagem de *debris* presente no terço apical dos canais foi calculada. Os dados foram submetidos à análise estatística por meio de ANOVA e teste de Tukey. Os resultados do presente estudo evidenciaram que não houve diferença estatisticamente significante ($p>0,01$) entre os grupos irrigados com NaOCl a 1% e detergente de mamona a 3,3%, que apresentaram menor porcentagem de *debris* no terço apical, 8,49% e 10,11% respectivamente. O grupo irrigado com água destilada e deionizada apresentou a maior porcentagem de *debris* (15,58%), sendo estatisticamente diferente ($p<0,01$) dos outros grupos experimentais. Concluiu-se que o detergente de mamona a 3,3% apresentou efetividade semelhante ao NaOCl a 1% na remoção de *debris* dos canais radiculares.
Unitermos: Limpeza dos canais radiculares; Instrumentação rotatória; Solução irrigante; *Ricinus communis*

INTRODUCTION

Cleaning of root canal system during biomechanical preparation aims to eliminate irritating agents, such as bacteria and their byproducts, degenerated pulp tissue and contaminated dentin[22]. This process occurs by the mechanical action the endodontic instruments on the root canal walls associated with the chemical properties of irrigating solutions and physical action of the flushing/aspirating process.

Organic and inorganic debris are left inside the root canals after biomechanical instrumentation[14]. The smear layer, composed of dentin chips, remnants of pulp tissue, odontoblastic processes, and sometimes microorganisms, is formed on the root canal walls as a result of chemomechanical instrumentation and is deposited mainly at the apical third[17].

Over the years, the development of several types of instruments and irrigating solutions have optimized the cleaning and shaping of root canals, thus increasing the safety and decreasing the duration of endodontic therapy.

Among the currently available irrigating solutions, sodium hypochlorite (NaOCl) at different concentrations is the most commonly used and accepted worldwide due to its properties of clarification, organic tissue dissolution, saponification, transformation of amines into chloramines, deodorizing and antimicrobial effects[2,22,23]. Although sodium hypochlorite is still the most used root canal irrigant, studies have searched for alternative solutions and therapeutic resources that may improve the success rate of endodontic treatment.

The development of polyurethane resins derived from castor oil (obtained from the bean produced by *Ricinus communis)* widened the scopes in different fields of medical and dental research, as they were shown to have biocompatibility and potential applicability in several areas[5,18].

The castor plant (*Ricinus communis;* division *Magnoliophyta*, class *Magnoliopside*, sub-class *Rosidae*, order *Euforbiales*, family *Euforbiaceae*) is a vegetal typically found in tropical climate areas. Although originally native to northeastern Africa and the Middle East, it has become naturalized in several tropical and warm temperate regions throughout the world, including Brazil. Because of its climatic characteristics, natural resources and territorial extension of continental dimensions, Brazil has one of the largest areas cultivated with castor bean plant in the world. The *Ricinus communis* has great oil-chemical potential that may guarantee the supply of prepolymers and polyols derived from fatty acid in large scale. Because of its composition, 81-96% of triglyceride of the ricinoleic acid, the castor bean oil is considered a natural polyol, since it has three hydroxyl radicals that are liable to be utilized in the synthesis of polyurethanes

Based on the outcomes of biocompatibility studies in Orthopedics[4,15], dental researchers have reported the viability of using materials derived from *Ricinus communis* for bone reconstruction and repair of intrabony defects, maxillary sinus floor elevation and filling of dental alveolus, in such a way to provide viable sites for placement of implants and metallic pins and frameworks[5,6].

In Endodontics, a detergent derived from the castor bean oil was developed for utilization as a root canal irrigating solution. This *Ricinus communis* detergent has similar antimicrobial activity as 0.5% sodium hypochlorite when used for irrigation of necrotic root canals[11,12], is biocompatible with the periapical tissues[6], increases dentinal permeability[19] and has similar ability to remove smear layer from the root canals as 17% EDTA[24].

Therefore, the purpose of this study was to investigate, by morphologic and morphometric analyses, the cleaning of root canals instrumented with nickel-titanium rotary system using 3.3% *Ricinus communis* detergent and 1% sodium hypochlorite as irrigating solutions.

MATERIAL AND METHODS

Twenty-seven human single-rooted mandibular premolars, with straight roots and no flattening (either in mesio-distal or bucco-lingual direction) were selected for this study. The teeth were washed in running water for 24 h and stored in distilled water until use.

Pulp chamber access was performed according to the guidelines recommended by De Deus[7] (1992) under irrigation with distilled water to avoid that dentin chips and other debris were taken into the root canal inadvertently.

The root canal was explored with a size 10 K-file (Dentsply/Maillefer, Ballaigues, Switzerland) until the tip of the instrument penetrated and engaged the apical foramen. The working length was determined at 1 mm short of this point.

The teeth were randomly divided into 3 groups (n=9) according to the irrigating solution used: Group I: distilled and deionized water (2 mL at each file change; 20 mL total); Group II: 1% NaOCl (2 mL at each file change; 20 mL total) and Group III: 3.3% *Ricinus communis* detergent (2 mL at each file change; 20 mL total).

Biomechanical preparation was performed with nickel-titanium rotary instruments activated by Endo Plusâ electric engine (VK Driller Equipamentos Elétricos LTDA, São Paulo, Brazil) adjusted to 250 rpm. The canal entrance was prepared with a 25/02 Endo-Flare instrument (MicroMega, Besançon, France), whereas the cervical and middle thirds were prepared with ProTaper (Dentsply/Maillefer, Ballaigues, Switzerland) S1, SX and S2 shaping files. Instrumentation was continued using the Hero 642 system (Micromega, Besancon, France) in the following sequence: 25/02 (apical), 25/04 (apical), 25/06 (middle), 30/02 (apical), 30/04 (apical) and 35/02 (apical). The size of the instrument was recorded and standardized for all specimens.

In all groups, at every change of instrument, the root canals were flushed with 2 mL of the respective irrigating solution using the Ultradent syringe (Ultradent, Endodontics Products, Texas, USA).

After biomechanical preparation, the organic debris remaining in the root canals were fixated by 12-hour immersion of specimens in 10% buffered formalin. Thereafter, the apical thirds were sectioned and removed for histological processing. The specimens were washed, decalcified in 10% trichloroacetic solution and embedded in paraffin. Transverse 6-µm-thick semi-serial sections were obtained and stained with hematoxylin-eosin for morphometric analysis.

The cross-sections were examined with an optical microscope (Eclipse E 600; Nikon, Shinagawa-Ku, Tokyo, Japan) at X40 magnification connected to a computer. The images were captured using Adobe Premier 5.1 software and analyzed using Corel Photo Paint 10 software. A grid was placed over these images to evaluate the total area of the canal and the area containing debris (Figure 1). After the count of the points in the clean area and the debris area, the percentage of debris in the root canal at the apical third was calculated. These data were submitted to statistical analysis with ANOVA and the Tukey test ($p<0.01$).

RESULTS

The percentages (means and standard deviation) of debris remaining in the apical thirds of the root canals in each group are shown in Table 1.

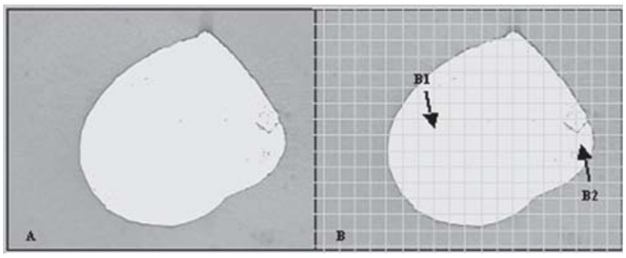

FIGURE 1- Microscopic image (X40) of the apical third. B. The same image with the grid; Arrows indicate areas without (B1) and with debris (B2)

The Tukey test was applied and the results showed no statistically significant difference ($p>0.01$) between the groups irrigated with 1% NaOCl and 3.3% *Ricinus communis* detergent, which had the lowest amount of debris at the apical third (Table 1; different letters indicate statistically significant differences) and the group irrigated with distilled and deionized water presented the greatest percentage of debris (15.58%) and was statistically different from the other groups ($p<0.01$).

DISCUSSION

Despite the noticeable technical and scientific advances recently experienced in Endodontics, which led to the development of a wide variety of instruments, equipments and materials, research has demonstrated that biomechanical preparation does not yield complete removal of organic and inorganic debris from instrumented root canals[1,10].

Biomechanical preparation of the root canal system with Ni-Ti rotary instruments driven by electric and pneumatic engines is a worldwide spread reality that has brought several conceptual changes and remarkably improved the effectiveness and rapidness of endodontic therapy. Nevertheless, while investigating the cleaning capacity of manual and rotary instrumentation techniques in mesio-distal flattened canals by morphometric analysis, Barbizam, et al.[3] (2002) found that rotary instrumentation with Ni-Ti files acts within a well-delimited area, leaving non-instrumented polar regions, in which the arrangement of dentinal tubules facilitates the entrapment and maintenance of viable microorganisms. It has also been pointed out that, even in canals with circular internal anatomy, the capacity of thoroughly cleaning the endodontic space does not depend exclusively on instrumentation[10]. These findings emphasize the need to use an effective irrigating solution that has specific chemical properties, among which organic tissue dissolution[2,16].

This study evaluated, by morphologic and morphometric

TABLE 1- Percentages of debris remaining at the apical thirds of root canals in each group

	H_2O	1% NaOCl	3.3% *Ricinus communis* detergent
	18.18	12.75	13.05
	13.58	3.72	5.46
	19.76	6.79	14.28
	17.68	5.82	13.32
	13.11	8.00	6.13
	16.40	11.68	8.93
	13.76	6.54	8.07
	15.26	8.20	10.94
	12.48	12.87	10.83
Mean ± SD	15.58 ± 2.53 a	8.49 ± 3.25 b	10.11 ± 3.17 b

Tukey test ($p<0.01$): Critical value = 0.33. Different letters indicate statistically significant difference analyses, the cleanliness of the apical third of root canals after NiTi rotary instrumentation associated with the use of either 3.3% *Ricinus communis* detergent or 1% NaOCl, as irrigating solutions. The percentage of debris at the apical third of root canals after chemo-mechanical preparation was assessed morphometrically, as described in previous studies[1,3,10,21].

The findings of the present study revealed that there was no statistically significant difference ($p>0.01$) between groups irrigated with 1% NaOCl and 3.3% *Ricinus communis* detergent, which had the lowest amount of debris at the apical third. These groups, in turn, were statistically different ($p<0.01$) from the control group irrigated with distilled and deionized water.

The results obtained for specimens irrigated with 1% NaOCl are probably derived from the sodium hypochlorite capacity to dissolve organic tissue remnants[2,9,13,23].

Estrela, et al.[8] (2002), while discussing the mechanism of action of sodium hypochlorite, reported that this chemical agent is able to produce alterations in cellular biosynthesis and changes in cellular metabolism and phospholipid degradation, by formation of chloramines that interfere with cellular metabolism, oxidation action that promotes irreversible bacterial enzymatic inhibition, and degradation of fatty acids and lipids.

The group irrigated with 3.3% *Ricinus communis* detergent during rotary instrumentation had similar results as those of the group irrigated with 1% NaOCl, regarding the removal of smear layer from root canals. It is worthy mentioning that the use of a *Ricinus communis*-based detergent as an endodontic irrigant is in compliance with the recommendations of the World Health Organization, which has encouraged the search for substances and products derived from animal, vegetal and mineral sources.

The results obtained in the group irrigated with the *Ricinus communis* detergent may be ascribed to its low surface tension and consequent moisturizing capacity, which allow for an intimate contact of the solution with the entire surface of root canal walls. After humectation, the adsorption phenomenon takes place, i.e., the hydrophobic portion (which is also lipophilic) binds to the fatty matter, and the hydrophilic portion binds to water. Therefore, the hydrocarbonated chain works as a structure that has fat bound to one end and water bound to the other. This configuration leaves the surface free from fatty (oil) contamination, protected by the detergent molecules attached to it[19]. As a result, the mechanical action of endodontic files during instrumentation increases the contact surface between the detergent and root canal walls, thus facilitating the removal of debris.

Studies have shown that, in addition to its ability of removing root canal debris, the 3.3% *Ricinus communis* detergent has antimicrobial activity[11,12] and smear layer removal capacity[24], increases dentinal permeability[20] and presents biocompatibility with the periapical tissues[5,6]. Because of the aforementioned properties and based on the outcomes of this study and previous investigations[11,12,20,24], it seems feasible to suggest that this intracanal irrigating solution derived from *Ricinus communis* may be a viable alternative for patients that have hypersensitivity to sodium hypochlorite.

In view of the issues discussed throughout this paper, and considering that the currently available endodontic irrigants do not have anti-inflammatory activity and biocompatibility, the authors' expectation with this study was to offer new perspectives and research-based information that might be helpful in evaluating whether the 3.3% *Ricinus communis* detergent, a natural product derived from a vegetal source, may be used as an irrigating solution during biomechanical preparation of root canals.

CONCLUSIONS

According to the methodology proposed and based on the results of this study, the following conclusions may be drawn:

1. None of the tested solutions yielded complete removal of debris from the apical third of root canals;
2. 3.3% *Ricinus communis* detergent and 1% NaOCl presented similar cleaning effectiveness on elimination of debris from the apical third of root canals.

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