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Analysis of *Aloe vera* cytotoxicity and genotoxicity associated with endodontic medication and laser photobiomodulation☆

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ABSTRACT

This study aims to evaluate, *in vitro*, the effect of *Aloe vera* associated with endodontic medication, with or without laser photobiomodulation (FTL) irradiation in FP6 human pulp fibroblasts. The materials were divided into eight groups: CTR - control; CL - FTL alone; AA - *Aloe vera* with distilled water; AL - *Aloe vera* with distilled water and FTL; HA - calcium hydroxide P.A. with distilled water; HL - calcium hydroxide P.A. with distilled water and FTL; HAA - calcium hydroxide P.A. with *Aloe vera* and distilled water; HAL - calcium hydroxide P.A. with *Aloe vera*, distilled water, and FTL. The cytotoxicity was evaluated by MTT assay at 24, 48, and 72 h and the genotoxicity by micronucleus test assay. This study was performed in triplicate. Data obtained in both tests were statistically analyzed by ANOVA and Tukey's tests ($p \leq 0.05$). Group AA presented high genotoxicity and low cytotoxicity. After 24, 48, and 72 h, the group HAA significantly reduced the cell viability. Interaction with FTL showed slightly increase cell viability after 24 and 48 h in groups CL and HL ($p < 0.001$), despite the high genotoxicity in group CL and low genotoxicity in group HL. Group AL showed higher cell survival rate at 72 h ($p < 0.05$) and high genotoxicity ($p < 0.001$). It was concluded that *Aloe vera* allowed higher cell viability in human pulp fibroblasts in the presence of calcium hydroxide or with FTL separately, but genotoxicity increased in these associations.

1. Introduction

The main objectives of root canal therapy are removal of pathologic pulp, cleaning, disinfection of contaminated root canals, shaping and obturation of the root canal system in three dimensions to prevent re-infection [1]. Hence, the professionals must know the complexity of the root canal system (RCS) of teeth with apical periodontitis and periapical radiolucency, to choose the better technical in these cases, to associate substances and medications that disinfect dentinal tubules, and to promote repair of periapical tissues [2].

The presence of microorganisms in areas with a difficult instrument access such as isthmus, ramifications, lateral canals, apical accessories and delta canals, and deep into dentinal tubules can interfere in the reparation process, thus requiring medication in between sessions to reach these areas [3].

Calcium hydroxide, antiseptics, and antibiotics are the most used medications in endodontic practice [4]. These medications can eliminate bacteria that survival to biomechanical preparation, reduce periradicular inflammation, neutralize toxic products, and stimulate repair by mineralized tissue [5].

Cytotoxicity is a destructive effect of some material on the cells [6], and genotoxicity is a detection of genetic material damages such as DNA and chromosome breakage, genetic mutation, and change in the repair ability of DNA [7]. In order to justify its applicability, intracanal medication should present high antimicrobial activity and low cytotoxicity and genotoxicity, aiming to achieve a potential for eliminating bacteria without damaging periapical tissues [8,9].

Laser photobiomodulation is another resource used in dental

☆ Conflicts of interest: none.

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practice, acting as analgesic, anti-inflammatory, anti-edematous, and helping the tissue repair process [10]. The mechanisms of laser therapy irradiation include an increase of local microcirculation, angiogenesis, vasodilation, and inhibition of inflammatory mediators such as prostaglandins. Laser irradiation can avoid initiating the arachidonic acid cascade on damaged tissues, decreasing prostaglandin production [11].

It has been performed studies of new natural products with repair abilities aiming to support and accelerate the endodontic treatment in teeth with apical periodontitis and periapical radiolucency. The *Aloe vera* plant popularly known as "babosa" features among these products, keeping interesting properties to endodontic [12] such as anti-inflammatory and antibacterial action, as well as tissue repair stimulation [13]. Therefore, this study aims to analyze the cytotoxicity and genotoxicity of *Aloe vera* associated with endodontic medication with or without laser photobiomodulation irradiation in human pulp fibroblasts at 24, 48, and 72 h.

2. Materials and Methods

The entire research method was performed according to the guidelines of the International Organization for Standardization (ISO) 10993-5:2009 [14] and in triplicate. The cell bank of the Laboratory of Cell Biology of the São Paulo State University (UNESP), Campus São José, Brazil, provided the human pulp fibroblasts (FP6) lineage used in the research.

2.1. Division of Experimental Groups

The intracanal medication used was calcium hydroxide P.A (Biodinâmica Química e Farmacêutica LTDA, PR, Brazil) associated with *Aloe vera*, with or without laser photobiomodulation irradiation. The vehicle selected for this experiment was distilled water (Eurofarma Laboratorios LTDA, SP, Brazil), due to its biologically inactive property. Table 1 shows the groups description and the concentrations of the intracanal medications used in this study.

FTL: † laser photobiomodulation.

2.2. Preparation of Extracts

The *Aloe vera* leaves were obtained in the state of Sergipe, identified by voucher number ASE-37.261. A parenchyma scraping provided the gel, which was filtered, stored in sterile collector, wrapped in plastic film, and kept between − 18° to − 25° until freezing. Then, the sample was lyophilized. The *Aloe vera* pastes were manipulated in sterile Becker, stored in Falcon tubes protected with aluminum paper, and maintained at room temperature for 24 h. The same tube received 5 mL of DMEM culture medium supplemented with 10% SBF and penicillin/streptomycin; and incubated in 5% CO_2 at 37 °C, for 24 h.

Table 1
Description of groups and the concentrations of the intracanal medications.

Groups	Substances	Concentrations
CTR [Control)	Fibroblast culture medium	200 µL/well
CL	FTL †	–
AA	*Aloe vera*	4%
	Distilled water	
AL	*Aloe vera*	4%
	Distilled water	
	FTL †	
HA	Calcium hydroxide P.A.	90.9%
	Distilled water	
HL	Calcium hydroxide P.A.	90.9%
	Distilled water	
	FTL †	
HAA	Calcium hydroxide P.A.	16.39%
	Aloe vera	3.27%
	Distilled water	
HAL	Calcium hydroxide P.A.	16.39%
	Aloe vera	3.27%
	Distilled water	
	FTL †	

Table 2
Irradiation parameters for groups CL, AL, HL, and HAL.

Irradiation parameters	
Emission mode (CW)	Continuous
Length	ʎ660 nm
Active medium	InGaAlP
Laser optical power (output)	10 mW
Laser optical power [(nput)	40 mW
Beam area	4 mm^2
Power density (PD)	1 mW/cm^2
Energy density (ED)	3 J/cm^2
Irradiation time (by session)	3 s
Total energy per session (by well)	0.12 J
Beam divergence perpendicular to the junction	17°
Tip angle	50°

The specimens were produced with standardized shape and volume for all groups. Hence, the pastes were inserted in 5-mm diameter and 2-mm thick sterile polyethylene tubes [14]. A humid atmosphere conditioned the calcium hydroxide pastes at 37 °C with 5% CO_2 for 24 h so that all specimens would begin setting. After this period, still inside the polyethylene tubes, the medication was sterilized by ultraviolet radiation in the laminar flow chamber for 1 h to avoid contamination [15]. The *Eppendorf* tubes received 1 mL of DMEM culture medium supplemented with 10% SBF and penicillin/streptomycin, and were stored at 37 °C and 5% CO_2 for 24 h for the active products of the intracanal medication could release through the culture medium, producing eluates [8,15].

2.3. Parameters for Laser Irradiation

Laser irradiation was performed by a Twin-laser device (MMOptics™, Equipamentos Ltda., São Carlos, São Paulo, Brazil). The device was registered at the Brazilian National Health Surveillance Agency (ANVISA) under # 80051420007 and certified by the Brazilian Institute of Metrology, Standardization, and Industrial Quality (INMETRO) under # NCC 2756/05. The plates were irradiated 1 h after wells contacting eluates, and again with a 6-hour interval [16]. Table 2 describes irradiation parameters.

The desired application per well was 3 J/cm^2. It was standardized the design of experiments and developed a laser-adapted support for placing the plates to be irradiated. Knowing that the distance between the laser and the application surface is crucial, the distance between the laser beam and the cells was standardized. It was intercalated the wells to avoid energy overload due to the spreading characteristics of laser irradiation. A dark mask made of perforated matte cardboard was placed on the plate according to the location of wells, exposure only the area to be irradiated. After laser irradiations, the plates were incubated again at 37 °C in humid atmosphere with 5% CO_2 [16].

2.4. Cytotoxicity Test

Human pulp fibroblasts (FP6) were cultivated in DMEM (LGC Biotecnologia, Cotia, Brazil) supplemented with 10% SBF (Invitrogen, New York, USA) at 37 °C and 5% CO_2. Therefore, 8×10^3 cells were cultivated with 4 mL of cell medium for 24 h at 37 °C in atmosphere of 5% CO_2, in plates of 96 wells (Prolab, São Paulo, SP, Brazil). The culture medium present in the wells of the plates where fibroblasts adhered was removed and added 200 µL of the eluate of medication tested to each well. After, the eluates were removed and added 100 µL of MTT (3-(4,5-dimethylthiazol-2-il)-2,5-diphenyltetrazolium bromide) (Life Technologies, Carlsbad, USA) reagent solution, in each experimental