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Research Article

An *In Vitro* Evaluation of Antibacterial Effect of *Morinda citrifolia* Irrigation Solution Activated by Laser on Root Canal

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Abstract

Aim: To evaluate bacterial reduction after using laser and *Morinda citrifolia* in root canals infected by *Enterococcus faecalis*.

Materials and Methods: Seventy human single rooted teeth are used in this study. After endodontic preparation. Sixty teeth were infected by 20µL *E. faecalis* (ATCC 29212) and specimens were incubated for 72 hours. Groups were formed according to the irrigation protocol used:

Group 1: Saline irrigation,

Group 2: Laser irrigation,

Group 3: Laser and *Morinda citrifolia* irrigation,

Group 4: *Morinda citrifolia* irrigation.

After disinfection procedures the samples isolated from root canals were placed into blood agar, incubated at 37°C for 24 h and then were counted for colony-forming units. One-Way ANOVA was used for the statistical evaluation of data. For multiple comparisons, Tamhane's T2 test was used.

Results: The highest bacterial colonization was observed in the Laser Group.

Morinda citrifolia with laser was best at eliminating *E. faecalis*.

Followed by *Morinda citrifolia* and Saline group.

Keywords: *Morinda citrifolia*; Laser; Irrigation; *E. Faecalis*; Root Canals

Introduction

Irrigation is an important part of successful root canal treatment as it fulfils several important mechanical, chemical and (micro) biological functions including the healing of periapical tissues. It is the only way to reach and impact those areas of the root canal wall which are not touched by mechanical instrumentation. The initial and primary endodontic treatment (root canal treatment) goal must be to optimize root canal disinfection and to prevent re-infection.

Enterococcus faecalis is an anaerobic gram-positive coccus it has demonstrated good adaptation to environments with rich nutrient and low oxygen levels and complex ecology. It has the ability to invade dentinal tubules and does not depend on the survival of other bacteria. It plays an essential role in persistent failure of endodontic therapy. To overcome these failures caused by bacteria, proper irrigation protocol helps prevail the treatment.

Ideal root canal irrigants should be biocompatible, nontoxic, and with a desirable smell and taste. Chemical irrigants, even though effective in root canal irrigation, are associated with several disadvantages. NaOCl causes allergic reaction, tissue toxicity, staining of instruments, irritation to periapical tissue, inability to remove smear layer, and has an undesirable smell and taste.

Various herbal extracts, such as neem and tulsi extracts, *Aloe vera*, *Morinda citrifolia*, curcum longa, and turmeric, having antimicrobial, antiinflammatory, and therapeutic effects are promising to be used as endodontic irrigants.

The potential side effects, safety concerns, and ineffectiveness of CHX and NaOCl to completely clean root canals suggest neither of these two irrigating solutions is ideal. Currently, none or few natural fruit juices that might be used as an alternative to NaOCl or CHX as an effective root canal irrigating solution have been identified. The literature has shown that *Morinda citrifolia* juice has antimicrobial and therapeutic effects [2].

It has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hy-

potensive, anti-inflammatory, and immune-enhancing effects. MCJ contains the antibacterial compounds L-asperuloside and alizarin. Acetone extracts from MCJ also demonstrated some antimicrobial activity [2].

Root canal disinfection is the primary challenge in endodontic procedures, and although the use of irrigants may decrease the number of microbes in infected root canals, they do not achieve the total disinfection of entire root canal systems. Lasers are one method used to overcome this challenge because lasers can access tubular systems that irrigants cannot.

The use of diode lasers in endodontics is an innovative approach to root canal disinfection because these lasers have the ability to penetrate deeper into dentinal tubules. Diode lasers have become the method of choice due to their advantages including their ease of use and relatively small size compared to other types of lasers. Disinfection with a combination of irrigants and diode lasers resulted in the greatest bactericidal effect. Therefore, the diode laser is considered as an adjunct to enhance the bactericidal effect of endodontic irrigants. Diode lasers combined with irrigants resulted in the highest root canal disinfection rate when compared with irrigants or lasers alone.

Methods

Sixty human single rooted teeth were used in this study. Mesiodistal and bucco-lingual direction radiographs were taken from the teeth to confirm the presence of a single canal. Teeth were decoronated and 14 mm long roots were obtained, approximately 14 mm in length. Roots were prepared up to protaper F3 file (Dentsply-Maillefer, Ballaigues, Switzerland). After each file, 2 mL of 5% NaOCl was used. 5 mL of 5% NaOCl, 5 mL of 15% ethylenediaminetetraacetic acid solutions and Cold Saline were used for the final irrigation respectively. Root canals were dried with paper points. Root apexes were sealed using composite resin. Specimens were placed into Eppendorf tubes with silicone impression material. Teeth were packaged one by one and sterilized in an autoclave at 121 °C for 30 minutes. After sterilization, 10 teeth were not infected to confirm the accuracy of the sterilization and were evaluated as a negative control group. *E. faecalis* cultures (ATCC 29212) incubation was done for 72 hours and were placed in Brain Heart Infusion broth medium. Solution was prepared to be 0.5 Mc Farland. Each sample was inoculated with 20 µL of the prepared solution and specimens were incubated at 37 °C in the incubator for 72 hours. For 10 samples no disinfection procedure was applied, which were evaluated as the positive control group.

Groups were formed as follows:

Group 1: Saline Irrigation
Root canals were irrigated with 10 mL of Saline

Group 2: Laser Irrigation

Root canals were irradiated for 1 minute with a 940 nm wavelength diode laser at 1W power utilizing the continuous mode with endodontic tips.

Group 3: Laser and *Morinda citrifolia* Irrigation
Root Canals were irrigated with 10mL of *Morinda citrifolia* solution and activated with diode laser for 1 minute.

Group 4: *Morinda citrifolia* Irrigation
Root Canals were irrigated with 10mL of *Morinda citrifolia* solution.

Three sterile paper points for each tooth were moistened with 0.5 mL of sterile distilled water and were placed into the root canal one by one for 1 minute. Paper points were placed into sterile Eppendorf tubes with brain heart infusion medium and specimens were sent to the microbiology laboratory. The Vortexing procedure was performed on samples and 20 µL of liquid medium was inoculated on blood agar plates. Specimens were incubated at 37 °C for 24 hours and the colony-forming units (CFU) were counted at the end of incubation.

Results

The highest bacterial colonization was observed in the Laser Group. *Morinda citrifolia* with laser was best at eliminating *E. faecalis*. Followed by Laser and *Morinda citrifolia* Irrigation group.

Discussion

Irrigation in addition to mechanical preparation is required in order to increase the efficiency of preparation and effectively eliminate bacteria from the root canal system.

These irrigation solutions should eliminate endodontic pathogens, persistent infection and resistant bacteria. For this purpose, many solutions and their various concentrations and different disinfection methods are used in clinics and research.

However, the cytotoxic properties of the solutions beside antibacterial activity have prompted researchers to seek other options.

Recently, new systems and materials have been proposed to replace the traditional chemomechanical process or supporting their impact to improve root canal disinfection.

Polynesians have used the fruit juice from the exotic *Morinda citrifolia* or noni plant in folk remedies for more than 2000 years. *Morinda citrifolia* juice (MCJ) has a broad range of therapeutic effects, including antibacterial, antiviral, antifungal, antitumor, anthelmintic, analgesic, hypotensive, anti-inflammatory, and immune-enhancing effects.

MCJ contains the antibacterial compounds L-asperuloside and alizarin. Acetone extracts from MCJ also demonstrated some antimicrobial activity.

The antimicrobial effects of natural fruit juices and plant extracts on *Enterococcus faecalis* and other endodontic pathogens have generally not been evaluated, except for Arctium lappa plant extract, which was effective at disinfecting root canals. A substantial number of bacterial species have been identified as inhabitants of the oral cavity. However, because of bacterial interactions, nutrient availability, and low oxygen potentials in root canals with necrotic pulp, the number of bacterial species present in endodontic infections is restricted.

These selective conditions lead to the predominance of facultative and strictly anaerobic microorganisms, particularly *E. faecalis*, which survive and multiply, causing infections that stimulate local bone resorption and are more resistant to endodontic treatment.

Cleaning is one of the main objectives of root canal preparation. Thorough cleaning removes microorganisms, permits better adaptation of filling materials, and enhances the action of intracanal medicaments.

The choice of an irrigant is of great importance because they might vary in their effectiveness to act as lubricants during instrumentation and flush debris, smear layer, and bacteria out of the canal. Different chemical formulations of irrigants might also have different reactions with pulp, necrotic tissues, and microorganisms [1-18].

Conclusion

The highest bacterial colonization was observed in the Laser Group. *Morinda citrifolia* with laser was best at eliminating *E. faecalis*. Followed by Laser and *Morinda citrifolia* Irrigation group.

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