ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

ANTIMICROBIAL EFFICACY OF DIFFERENT HERBAL EXTRACTS AS INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS

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ABSTRACT

Introduction: A successful endodontic treatment depends upon complete debridement of microflora from the root canalsystem. However, due to complex root canal configuration, complete debridement through mechanical instrumentation alonecannot remove entire bacterial load. So,the aim is *in vitro* evaluation of antimicrobial efficacy of biological extracts against*Enterococcus faecalis* MTCC-439 strain when used as intracanal medicaments. The medicaments used were Nissin, anantibiotic peptide; calcium hydroxide, curcumin and aloe vera

Materials and Methods: Eighty single rooted lower premolar teeth which were extracted for orthodontic purpose were collected.Toothspecimens were sectioned at cement-enamel junction with a diamond saw to obtain a standard root length.

Complete biomechanical preparation will be done, which will be followed by inoculation of E.faecalis

After inoculation, the samples will be kept in a closed eppendorf tube and incubated at 37°C for 21 days under aseptic conditions. The canals will be re-inoculated with fresh bacterial samples at every 3 days interval to ensure viability of bacteria.

The canal contents will be aspirated after 21 days of incubation, then rinsed with 5 mL saline and patted dry with sterile paper points. The specimens will be then randomly divided into following groups:

Group 1: Calcium hydroxide

Group 2: Aloe vera

Group 3: Curcumin

Group 4: Nisin Calcium hydroxide.

Results: In present *in vitro* study, Nisin showed no CFU while aloe vera and curcumin showed significantly less growth ascompared to calcium hydroxide against *E. faecalis*.

Conclusions: Nisin outreach curcumin and aloe vera in eliminating the *E. faecalis* when used as intracanal medicaments.

INTRODUCTION

A successful endodontic treatment depends upon completedebridement of microflora from the root canal system.[1]However, due to complex root canal configuration,

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completedebridement through mechanical instrumentation alonecannot remove entire bacterial load.

Contemporarily use of chemical intracanal irrigators andmedicaments are requisite to debride infected tissues anderadicate microorganisms from the root canal system.[2]Moreover, low oxygen tension, less nutrient availability,and enormous bacterial interactions lead to predominantcolonization of facultative anaerobic species prevailing inthe root canals.[3] In persistent peri radicular infections,*Enterococcus faecalis* had been isolated in about 24%–77%cases that perpetually resulted in failure of root canaltherapy. This could be because of the ability of *E. faecalis*to survive at high alkaline environment and deeper tubularinvasion. It grows through adhering on biofilm and colonizeson to the surface.[4]

Calcium hydroxide is a gold standard traditionally when usedas intracanal medicaments. Although its antibacterial activityis on wide range of microflora of the root canal, but wasfound less effective against *E. faecalis*. [5-8] Moreover, increasingrates of cytotoxic reactions and inability to eliminate themicroorganism from dentinal tubules by commerciallyavailable medicaments had laid a need of introduction ofnovel molecules used as intracanal medicaments. In lastfew decades, the use of alternative therapeutic agents hadconsiderably increased and are derived from plants, insects,microorganisms, etc.[1,3,9]

A natural occurring peptide isolated from strains of *Lactococcuslactis* termed as Nisin had been recently introduced. Thisantibiotic peptide is a Class I bacteriocin. It is effective againstGram-positive bacteria and spores[7,3] including strains of*E. faecalis*.[10] Nisin intensively used as a food preservative inover 40 countries and is safe to humans. Recent documentsare suggestive that nisin is effective in eradication of *E. faecalis*from root canals.[11]

Aloe vera(*Aloe barbadensis*miller) belongs to the Liliaceae family and is a cactus-like plant.[12] It is a natural medicament with a long history of usage in medicine and nutrition. The antimicrobial properties of *A. vera* against various species of microorganisms, including *E. faecalis*, have also been reported as *A. vera* has potent antibacterial, antiviral, and antifungal activities.[12,14] The possible reason for antimicrobial action of *A. vera* could be the presence of 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids, and amino acids.[15] The antimicrobial activity of *A. vera* might be attributed to the presence of carvacrol and thymol, and they are natural monoterpenes that act on the cell membrane of the organisms causing cellular death.[16]

Curcumin is a plant-derived agent (turmeric root), which also exhibits antioxidant and anticancer effects, thus having significant clinical relevance related to the prevention and treatment of numerous illnesses Curcumin has already been used in the fabrication of electro spunfibres for biomedical applications (e.g., skin tissue regeneration), and more recently, it was employed as an intracanalirrigantsduring endodontic treatment, showing effective and promising disinfection results , probably explained by its permeabilization effects that cause damage of bacterial membranes

Thus, the aim of present study is to compare antimicrobialproperties of various biological extract Nisin, curcumin, aloe vera as an intracanal medicament when compared withcalcium hydroxide.

SUBJECTS AND METHODS

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

The medicaments used in the study were calcium hydroxidepowder, Nisin (2.5%, Sigma Aldrich, USA),curcumin powder was dissolved in ethanol to obtain a stock solution (100 mg/mL). Materialsused were absolute ethanol, brain heart infusion broth,trypticase soy blood agar plates, and *E. faecalis* (MTCC-439)strain.

Literature survey

1. Study of various articles related to bacterial leakage.
2. Study in detail about biomechanical preparation, intracanal medicaments and various herbal extracts and its uses.
3. The existing approaches to perform degree of infection in root canal wall.

Simulation

1. Effect of various intracanal medicaments in root canal treatment.
2. Composition of herbal extracts.
3. Analysis of the leakage values

Experimental setup

Single canal roots, morphological similarities with no previous root canal treatment taken.

Complete biomechanical preparation will be done, which will be followed by inoculation ofE.Faecalis.

After inoculation, the samples will be kept in a closed Eppendorf tube and incubated at 37°C for 21 days under aseptic conditions. The canals will be re-inoculated with fresh bacterial samples at every 3 days interval to ensure viability of bacteria.

The canal contents will be aspirated after 21 days of incubation, then rinsed with 5 mL saline and patted dry with sterile paper points. The specimens will be then randomly divided into following groups:

Group 1: Calcium hydroxide

Group 2: Aloe vera

Group 3: Curcumin

Group 4: Nisin

Study and comparison

Eighty single-rooted lower premolar teeth which will be extracted for orthodontic purpose will be collected. Tooth specimens will be sectioned at cement-enamel junction with a diamond saw to obtain a standard root length. The specimens will be randomly divided into four groups (n = 20). The working length measurement will be done by measuring file length 1 mm less until tip of file was visible at the apical foramen. To standardize, the root will be cut to make working length as 10 mm. The root canals of the specimen will be instrumented with Protaperrotary files. After that copious intracanal irrigation will be done with 2.5% sodium hypochlorite and ethylenediamine tetra acetic acid (17% w/v) using 5 mL luer lock

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syringe followed by irrigating with 0.9% normal saline solution (Marck Biosciences, India) to remove smear layer.

Root Canal infection-

- Each root canal will be inoculated with 24 h old cultured broths of the bacterial solution up to the canal orifice using a sterile endodontic needle in a microbiological safety cabinet.
- After inoculation, the samples will be kept in a cotton plugged test tube and incubated at 37°C for 21 days. Every 3rd day, the canals were reinoculated with fresh bacterial samples. The canal contents will then be aspirated after 21 days of incubation, then rinsed with 5 mLsaline and patted dry with sterile paper points. The specimens will then be randomly divided into four groups (n = 20 each) for intracanal medicaments:
- The specimens will be divided into four groups which will be as follows-
    - Group 1: Calcium hydroxide
    - Group 2: Aloe vera
    - Group 3: Curcumin
    - Group 4: Nisin
- Preparation of calcium hydroxide mix-calcium hydroxide intracanal medicament is prepared by mixing powder with normal saline in the ratio of 1:1 to obtain the paste.
- Aloe vera gel (100% Aloe vera gel, Sillaneh Co., Iran).
- Preparation of aqueous Nisin - 200 mg/mL concentration of Nisin was prepared by dissolving it in distilled water.
- In all the samples, the prepared medicaments of 5 µL will be injected in the root canals and completely filled. The canals will then be sealed with sticky wax (Pyrax, India) and incubated at 37°C for 7 days. After 7 days of incubation, the wax will be removed from each of the canal entrances. The bacterial samples from each canal will be retrieved with sterile paper points and inoculated on brain-heart infusion broth (HiMedia Laboratories, India) and incubated at 37°C for 24 h. After that, each canal will be irrigated with 5 mL of saline and sterile paper points will be used to dry the canal.
- To evaluate the degree of infection of the canal wall and its radicular dentine, specimens ofdentine chips will be retrieved. The dentin chips will be then transferred by placing files into 0.5 mL of brain-heart infusion broth through sterile Eppendorf tubes and incubated at 37°C for 24 h. After 24 h, 5 µL. of solution will be inoculated on TSY-blood agar plates from each tube and incubated at 37°C for 24 h to obtain bacterial colony forming unit (CFU) count. Statistical analysis will be done using Kruskal Wallis test

RESULTS

The means CFUs count of the present study showed Nisin with no CFUs of *E. faecalis*. Curcumin,and Aloe vera can be used as an effective alternate intracanal medicament and had low CFU count as compared to calcium hydroxide alone.Statisticallysignificant

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differencewas present in CFU count when different medicaments usedagainst *E. faecalis* by Kruskal–Wallis test.

DISCUSSION

Formerly since a decade or more intracanal medicamenthad been used as an interim appointment dressing. Thecommonly used commercial synthetic medicaments arecalcium hydroxide, phenolic compounds (eugenol andcamphorated monochlorophenol), aldehydes (formocresol),halides (iodine potassium iodide), antibiotics, etc.[2] However,majority had been reported for its toxic effect, developmentof resistant strains and depletion of immune response. Thishad led the shift in paradigm from synthetic to naturallyderived medicaments.

E. faecalis is a Gram-positive coccus, been a facultativeanaerobic, it can penetrate deeper inside the dentinal tubuleswhich could possibly results in root canal reinfection.[17] Itresist chemico-mechanical instrumentation and can survive asa monoculture inside the root canals without any mutualismwith other bacterias. *E. faecalis* can even withstand highalkaline pH during calcium hydroxide dressing.[18] Thus,rendering a need of an alternative method for eradicating thisspecies and will be beneficial for the prognosis of endodontictreatment.

The present study showed Nisin as the most effectivemedicament against *E. faecalis* as there were none of theCFUs with Nisin. Nisin is an antimicrobial peptide, naturallyoccurring produced by *Streptococcus lactis*. It is commonlyused as a food preservative in meat and dairy industry.Previously, studies had reported the antimicrobial activityof Nisin against *E. faecalis* both *in vitro* and *in vivo*.[12] Nisinexerts its bactericidal activity through pores formation byinteracting with a specific molecule “Lipid II” and inhibit cellwall synthesis. The principal component of Gram-positivebacterial cell membrane is Lipid II. At a nanomolecular level,Nisin target this Lipid II as a “docking molecule” to formpores on the cell membrane surface and effectively killsthe bacteria.[19,20] Turner *et al*. showed significantly lowerinfected dentinal shaving of *E. faecalis* with Nisin whencompared to calcium hydroxide.[11]

Calcium hydroxide was ineffective against *E. faecalis* in thepresent study. The results were in accordance with the studiesdone by Harrison *et al*. and Hemadri *et al*.[21,22] *E. faecalis* isresistant to highly alkaline environment due to the presenceof proton pump as a primary resistance mechanism.[23] At pHof 11.5 *E. faecalis* cannot survive, however calcium hydroxidemedicament result in alkalinity of pH 10.3 within radiculardentine as being reported *in vitro*.[24] This is due to bufferingcapacity of dentine which provides a decreasing pH frominner to peripheral root dentine.[23]

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