

Grapefruit Seed Extract as an Alternative to Eliminate *E. Faecalis* in No Instrumented Endodontic Technique

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Abstract

No instrumentation endodontic treatment (NIET) is a new therapy to treat primary dental organs using a mix of different antibiotics to eliminate microorganisms present in necrotic pulp tissue. Until now, there are several publications that show promising clinical results with this technique but is still controversy the use of this drugs may cause bacterial resistance; for this reason, we consider that the use of substances of natural origin could be a new option.

Objective: To evaluate the *E. faecalis* inhibition of grapefruit seed extract and their capacity to potentiate the antimicrobial effect of CTZ paste.

Materials and methods: Different natural vehicles were evaluated alone and mixed with CTZ (propylene glycol, grapefruit seed extract, eugenol and as negative control 0.9% saline solution) A clinical isolated of *E. faecalis* was cultivated and distributed throughout plates with Müller Hilton agar to place a triplicate of absorbent paper disks embedded with the different vehicles and the mixes and incubated for 24 h at 37 °C. Data were analyzed and One-way ANOVA and Tukey test were used with a significance level of $p < 0.05$.

Results: For only vehicles, grapefruit seed extract presented the greatest inhibition with a halo of 30.9 mm showing a statistically significant difference with the rest of the vehicles. When vehicles were mixed with the CTZ paste, propylene glycol showed a greater inhibition with a statistically significant difference with the rest of the vehicles.

Conclusion: To the inhibition of *E. faecalis* could be possible the introduction of natural products in the application of NIET.

Keywords: Non instrumented endodontic technique; CTZ; grapefruit seed extract; *E. faecalis*

Introduction

Dental caries is the most prevalent oral disease in children, if this bacterial infection is not treated rapidly could involve dental pulp causing necrosis. To eradicate this polymicrobial infection, a has been proposed the noninstrumentation endodontic treatment (NIET) using a mixture of antibacterial drugs placed over the pul-

par floor [1] with the objective to prevent over instrumentation of root canals and irritation of periapical tissue decreasing chair time to only one visit [2]. In this context, CTZ (chloramphenicol, tetracycline, zinc oxide and eugenol) paste has been suggested for treatment of deciduous teeth with pulp necrosis [3]. Although this and other mixes of antibiotics have shown promising results [4,5] the

risk of hypersensitivity and increment of bacterial resistance lead us to evaluate natural origin alternatives with the capacity to eliminate microorganisms as *Enterococcus faecalis* (*E. faecalis*), a gram positive bacteria resistant to endodontic treatment [6] for their capacity to invade dentinal tubules, resist nutritional deprivation [7], as well as, delayed penetration of antimicrobial agents by the presence of its enterococcal surface protein (Esp) [8]. Grapefruit seed extract is a natural product obtained of *Citrus paradisi*, grinding their seeds, pulp and white membranes mixing them with glycerin [9]. This extract has shown a powerful antimicrobial activity [10] mainly attributed to the presence of polyphenolic compounds, flavonoids, citric and ascorbic acid, tocopherol and limonoid [11]. Propylene glycol (1,2-propanediol), a dihydric alcohol [12], has bactericidal activity [13], as a vehicle has demonstrated to improve the diffusing property of medicaments [14]. Therefore, the aim of this study is to evaluate if these natural products inhibit *E. faecalis* by themselves and have the capacity to potentiate the antimicrobial effect of CTZ paste.

Methodology

A clinical isolate was cultivated and characterized through PCR assay, confirming the presence of *E. faecalis*; this was maintained at standard conditions. The CTZ (Farmacia Galenico, Mexico) was commercially obtained to avoid the variation in the formulation and the presence of excipients. The evaluated natural products were 46% grapefruit seed extract (Nutribiotic, Mexico), 99.5% propylene glycol (Harleco, Mexico), as positive control 37.5% eugenol (Viarden, Mexico) and as negative control 0.9% saline solution

(PISA, Mexico). *E. faecalis* was adjusted to 0.5 McFarland standard, a bacterial suspension of 100 µl was distributed throughout Müller Hilton agar (Becton Dickinson, USA) to place a triplicate of absorbent paper disks of 5 mm in diameter impregnated with 5 µl of the different natural products and incubated 24 h at 37 °C. To evaluate if these natural products potentiate the antimicrobial effect of CTZ paste, 500 mg of CTZ were mixed with 400 µl of different natural products to obtain a paste with a firm and adhesive consistency as clinically applied. Inoculated plates with a triplicate of absorbent paper disk impregnated in each mixture were incubated 24 h at 37 °C. After incubation period inhibition zone was measured in millimeter with a digital vernier (CALDI-6MP, Truper). For the data analysis t-test and normality Shapiro-Wilk test were used with a significance level of $p < 0.05$ using Sigma Plot Software, version 11 (Systat, Software Inc.).

Results

Eugenol was used as a positive control to evaluate the bacterial inhibition increment against *E. faecalis*. In this study grapefruit seed extract showed the highest inhibition activity (2.17 times of increment), which had a statistically significant difference ($p < 0.05$). On the other hand, propylene glycol showed a negative value (-0.03) for bactericidal activity increment compared with eugenol solution (Table 1). Results of combined preparations showed a slight increase in bactericidal activity with CTZ + grapefruit seed extract compared with CTZ + eugenol, which is the vehicle normally used for the application of this paste (Table 1). CTZ + propylene glycol has a statistically significant difference ($p < 0.05$).

Table 1: Comparison of bacterial inhibition between the different products tested against *E. faecalis*.

Materials & controls	Mean ± SD	Bacterial Inhibition Increment*
Eugenol (positive control)	14.2 ± 0.3	1
Grapefruit seed extract	30.9 ± 3.6 [§]	2.17
Propylene glycol	13.8 ± 0.8	-0.03
Saline solution (negative control)	0.0 ± 0.0	-----
Combined preparations		
CTZ + Eugenol (positive control)	30.8 ± 0.6	1
CTZ + Grapefruit seed extract	31.0 ± 0.7	1.006
CTZ + Propylene glycol	36.9 ± 1.5 [§]	1.198
Saline solution (negative control)	0.0 ± 0.0	-----

*Increment of bacterial inhibition: times of bacterial inhibition.

§Statistical difference with the rest of the groups.

CTZ: Chloramphenicol, tetracycline and zinc oxide paste.

Discussion

Our findings suggest that grapefruit seed extract could be used directly without combining with other materials, could even be used in the cleansing phase in endodontic procedures in pri-

mary and permanent teeth that involve periodontal tissues without side effects since is biocompatible. This green technology extracts are already being evaluated as future option as intracanal irrigants, fruit extracts such blueberry and wild strawberry [15] and the obtention of enzymes from the peel of pineapple, orange and papaya

[16] has been proposed as an alternative to the use of sodium hypochlorite, avoiding damage and toxic effects caused by the use of this substance. In relation to combined preparations results, propylene glycol showed better results, this vehicle is broadly used in combination with antibiotics such as tri-antibiotic paste (TAP: ciprofloxacin, metronidazole and minocycline) to eliminate complex root canal infections [17–19]. Our results coincide with previous reports of clinical studies where the combination of this vehicle with antibiotic pastes shown radiographical and clinical success [20], mainly for the bacterial elimination in the area. We suggest that propylene glycol could be an excellent vehicle for CTZ as it could increase the clinical success of this paste. Although NIET proposes the use of topical antibiotic mixtures to treat deciduous teeth with chronic infection, these pastes may develop antibiotic resistance or cannot be used in resistant patients, therefore other medicament options should be explored. Grapefruit seed extract could be a promising option, their natural origin makes it biocompatible and in the same time this single solution has antimicrobial capacity against *E. faecalis*, a very resistant bacteria present in the oral microbiome associated with active dental infections, highly associated with failure in endodontic treatment of primary teeth [21].

Conclusion

The present study shows that grapefruit seed extract has antibacterial activity against an endodontic clinical isolate of *E. faecalis*; we conclude that this extract could be a future excellent natural option for NIET.

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