An All-inclusive Estimation of Antibacterial and Antifungal Efficiencies of Propolis and Cetrimide Root Canal Irrigants against *Enterococcus* faecalis and *Candida albicans*: An *In vitro* (Original Research) Study

Sumita Giri Nishad

Department of Conservative Dentistry and Endodontics, Santosh Deemed to be University, Ghaziabad, Delhi-NCR

Abstract

Background: This study was undertaken to rationally evaluate antibacterial and antifungal efficiencies of propolis and cetrimide root canal irrigants against *Enterococcus faecalis* and *Candida albicans*. **Materials and Methods:** Sixty extracted maxillary central incisors were chosen as study samples. Following ethical approval, all sample teeth were sectioned in their cervical region and intraoral periapical radiographs were taken for each tooth root for correct working length measurement. Root canals were infected by suspensions of *E. faecalis* and *C. albicans* and incubated for three days. All 60 samples were analyzed into three groups of 20 each (Group I: Propolis, Group II: Cetrimide, and Group III: Distilled water). Standard microbiological samples were obtained from all samples and stored independently. The total number of colony-forming units of tested microbial was estimated by routine microscopic procedures. **Results:** Resultant data were subjected to statistical analysis wherein $P < 0.05$ was considered significant. Group 1 sample was irrigated with propolis, Group 2 samples with cetrimide, and Group 3 with distilled water (control). Mean Group I bacterial load was 2.28 ± 0.02 for *C. albicans*. This was 2.96 ± 0.17 and 8.01 ± 1.91 for Group II and Group III, respectively. Mean Group I bacterial load was 2.63 ± 0.82 for *E. faecalis*. This was 2.88 ± 0.12 and 7.92 ± 1.42 for Group II and Group III, respectively. P value was highly significant for both the studied microbial. **Conclusion:** Within the limitations of the study, authors concluded that both propolis and cetrimide were effective against *C. albicans* and *E. faecalis*. Both have revealed satisfactory antimicrobial and antifungal efficacies against these microbial strains. Comprehensive evaluation confirmed that propolis was more effective than cetrimide in inhibiting growth of bacterial and fungal colonies residing inside root canals.

Keywords: Antimicrobial, *Candida albicans*, Cetrimide, *Enterococcus faecalis*, Propolis

INTRODUCTION

Literature has well evidenced that *Enterococcus faecalis* (*E. faecalis*) is the most commonly seen microorganism in active root canal infections. *E. faecalis* have few potent contaminating and infectious factors that easily attack dentinal tubules.[1,2] Such processes usually hamper normal nutritional activities of dental hard and soft tissues. One of the most popular ways of managing these dilemmas is to irrigate the root canal with antimicrobial agents. Over the years, researchers have studied several root canal irrigants with satisfactory antibacterial and antifungal activities. In any standard endodontic treatment, irrigation is integral part of biomechanical preparation and it must be augmented with certain medicament too.[3,4] Bacteria and fungus are usually identified as extremely complex and organized biofilms attached to the canal walls. Many of the popular studies showed efficient irrigation of root canals and accordingly satisfactory antibacterial results.[5,6] Therefore, overall success rate of the endodontic therapy (with irrigant) has increased and acceptable. Many of the microbiological studies confirmed that *E. faecalis* is an anaerobic Gram-positive coccus that may withstand complex atmospheric conditions.[7,8] *E. faecalis* is also capable to tolerate alkaline pH, waterless environment, and high concentration of salts.

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Address for correspondence: Dr. Sumita Giri Nishad, Department of Conservative Dentistry and Endodontics, Santosh Deemed to be University, Ghaziabad, Delhi-NCR. Phone: 9810400493. E-mail: sgiri_2000@yahoo.com

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Many of pioneer workers have confirmed that *E. faecalis* is the most common bacterial species identified endodontically treated teeth.[9,10] Furthermore, irrigation is an significant process for eradicating both morphological forms of bacteria (planktonic and biofilms). The overall efficiency of irrigation depends on multiple host related factor. Ideally, root canal irrigants must have a broad antimicrobial activity so as to cover anaerobic and opportunistic bacterium.[11,12] Literature has discussed several root canal irrigants including EDTA-, NaOCl-, and hexidine-based solutions. All have different antimicrobial and antifungal coverage and capacities. Most of them have highlighted only antimicrobial role of irrigating solutions.[13,14] In addition, *Candida albicans* also known to infect the canal milieu similarly like *E. faecalis*. In light of the above intermingling facts, this study was undertaken to rationally evaluate antibacterial and antifungal efficiencies of propolis and cetrimide root canal irrigants against *E. faecalis* and *C. albicans*.

MATERIALS AND METHODS

This study was designed, planned, and conducted in the department of conservative dentistry and endodontics of the institute. Author endeavored to assess antibacterial and antifungal efficiencies of propolis and cetrimide root canal irrigants against *E. faecalis* and *C. albicans*. Extracted maxillary central incisors were chosen as study samples with no signs of fracture, caries, attrition, erosion, and abrasion. Samples with any developmental anomaly were discarded. A total of 60 samples were evaluated and categorized into three study groups. All samples were collected from nearby clinics in the past 1 year and preserved as per standard norms. History revealed that the samples were from both male and female patients, however, author did not plan to interpretate the results as per gender. Mostly the teeth were removed due to periodontal or orthodontic reasons. Therefore, the study was conducted on *in vitro* basis only. At first, study synopsis was prepared and presented to the Institutional Ethical Committee for approval. Following approval, all sample teeth were cut in their cervical region by diamond disc driven by high velocity air rotor headpiece. Intraoral periapical radiographs were taken for each tooth root for correct working length measurement. Biomechanical preparation of the proposed root canals (40 K sized file only) was completed exactly as quoted in the standard literature and textbooks by distinguished workers. After root canal preparation, the apical foramen was sealed by self-curing acrylic resins. Since the microbial load was intended to be quantified, all prepared root canals were thoroughly sterilized. To infect the canal with *E. faecalis* and *C. albicans*, the related bacterial and fungal suspensions were inserted into the canal and incubated for 3 days. Antibacterial and antifungal activities of two commercially available root canal irrigants were evaluated (with distilled water as control). All 60 samples were divided into three groups of 20 each [G I: Propolis, G II: Cetrimide, and G III: Distilled water (control)]. Samples were irrigated with their respective irrigants carefully. Standard microbiological samples were obtained from all samples and stored separately as per their groupings. The total number of colony-forming units of tested microbial was estimated by regular microscopic procedures. Results thus obtained were subjected to statistical analysis wherein $P < 0.05$ was considered statistically significant.

STATISTICAL ANALYSIS AND RESULTS

Statistical analysis was completed using Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL, United States of America) statistical analysis software. Mean and standard deviation were calculated for intended parameters. The finalized data were subjected to suitable statistical tests to obtain *P* values, mean, standard deviation, Chi-square test, standard error, and 95% confidence interval. Response assessment and analysis demonstrated few very striking results. Author has discussed the clinical applicability of these inferences with other studies. Group 1 sample were irrigated with propolis, Group 2 samples with cetrimide, and Group 3 with distilled water (control). Table 1 shows types of irrigating solutions used in study as per the groups and no. of samples. Table 2 shows detailed statistical analysis and group-wise mean bacterial colony counts. Mean Group I bacterial load was 2.28 ± 0.02 for *C. albicans*. This was 2.96 ± 0.17 and 8.01 ± 1.91 for Group II and Group III, respectively (Graph 1). Mean Group I bacterial load was 2.63 ± 0.82 for *E. faecalis*. This was 2.88 ± 0.12 and 7.92 ± 1.42 for Group II and Group III, respectively. *P* value was highly significant for both the studied microbial (0.02 and 0.01 for *C. albicans* and *E. faecalis,* respectively). Table 3 shows comparison among the three study groups using one-way ANOVA. Level of significance (*P* value) was highly significant (0.001) for comparison conducted between groups.

DISCUSSION

Infection is one of the most unique diseases found in the human body. It is frequently associated with signs and symptoms of inflammations. In general, infection of any hard or soft tissue is initiated by microorganisms, especially bacteria.[15,16] In root canal spaces, few inhabitants microbial are being noticed very frequently and occasionally they initiate infections also. These are species of *Streptococcus, Enterococcus, Candida*, etc. *E. faecalis* is the most frequently seen species from dental infections including periodontitis, infected root canals, and periapical abscesses.[17,18] These are also very frequently found in the root canals of failed endodontic therapy. Studies have confirmed that *E. faecalis* has the capability to change

Table 1: Types of irrigating solutions used in study as per the groups and no. of samples

Groups	Group I	Group II	Group III
Irrigating solutions	Propolis	Cetrimide	Sterile distilled water (control)
No. of samples	20	20	20

Table 2: Detailed statistical analysis and group-wise mean bacterial colony counts

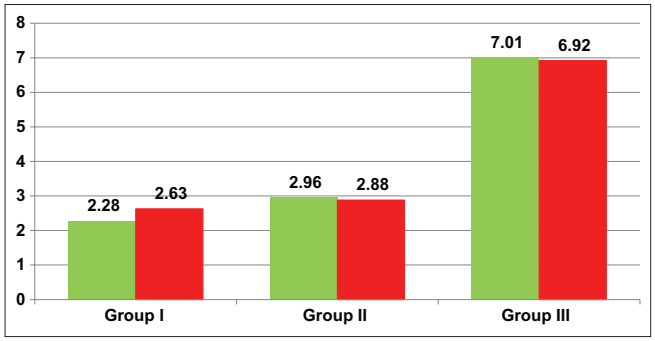
Microbial species	Mean (G I)	Mean (G II)	Mean (G III)	Std. deviation	Std. error	95% CI	Pearson Chi-square	*P*-value
Candida albicans	2.28±0.02	2.96±0.17	8.01±1.91	0.820	0.823	1.96	1.402	0.02*
E. faecalis	2.63±0.82	2.88±0.12	7.92±1.42	0.524	0.410	1.62	1.142	0.01*

*P<0.05 statistically significant. CI: Confidence interval

Table 3: Comparison among the three study groups using one-way ANOVA (for Groups I, II, and III)

Variables	Degree of freedom	Sum of squares Σ	Mean sum of squares mΣ	F	Level of significance (*P*-value)
Between groups	3	1.049	1.029	2.671	0.001*
Within groups	12	3.039	0.870		-
Cumulative	71.43	9.847		-	

*P<0.05 statistically significant



Graph 1: Microbial colony counts group wise (as per mean values)

it morphology for survival in a different of unfavorable environments.[19,20] Therefore, it can easily grow in the dead spaces of root canals with almost nil nutrients. This led to the exploration of efficient root canal irrigating solution which can eradicate these microbial. The clinical management of infected root canals by irrigating solutions is very critical and it also governs the long-term success of an endodontic therapy.[21,22] Researchers have also proposed many sequences or combination of multiple irrigants to make the canal completely sterilized. Many other experiments and approaches also tried to overcome this dilemma.[23,24] In our study, author endeavored to assess antibacterial and antifungal efficiencies of propolis and cetrimide root canal irrigants against *E. faecalis* and *C. albicans*. Literature has confirmed that the basis of successful endodontic treatment is to completely eradicate endodontic microbiota.[25,26] Biomechanical preparation is attempted for the removal of microbial by mechanical shaping of the root canal walls facilitated by irrigation with antimicrobial solutions. Traditional root canal irrigants like saline are usually ineffective in removing bacterial biofilms from the root canal walls. This literally initiates the development of pulpal and periapical disease.[27,28] Therefore, modern endodontic therapies are aimed to eliminate all microorganisms with minimal chances of reinfection. Many of the clinical researches have confirmed that biomechanical preparation and use of antimicrobial irrigants are helpful in diminishing the bacterial load in root canal milieu.[29,30] Studies on the failed endodontic therapies confirmed that root canals (without antimicrobial irrigation) usually show strains of many microbial including streptococci, lactobacilli, *Actinomyces, Candida*, and the Gram-positive bacteria species *E. faecalis*. Researchers have further confirmed that in cases of secondary root canal infections, the incidence of *E. faecalis* can reach up to 68%.[31,32]

CONCLUSION

Within the limitations of the study, author concluded that both of the studies irrigating solutions (propolis and cetrimide) were effective against *C. albicans* and *E. faecalis*. They have shown acceptable antimicrobial and antifungal efficacies against these microbial strains. Detailed assessment confirmed that propolis was more effective than cetrimide in inhibiting growth of bacterial and fungal colonies. Hence, propolis could be clinically utilized as an effective root canal irrigant to eradicate all microorganisms from canal. Our study results should be very carefully applied for similar clinical conditions since author has not included many of the interrelated factors. Furthermore, authors expect some other large-scale studies to be conducted that can further establish certain genuine and concrete guidelines in these perspectives.

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