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Original Article

The comparison of antibacterial effect of propolis , sodium hypochlorite 5.25%, and chlorhexidine 2% as intracanal irrigants against enterococcus faecalis: an ex vivo study

Maryam Zare Jahromi [1], Arezoo Tahmoorespoor [2],Nadia Hemmat [3], Elham Moghadasi Broujeni [3], Parisa Ranjbarian[4]✉

1. Assistant Professor, Department of Endodontics, Faculty of Dentistry, Islamic Azad University Isfahan (Khorasgan) Branch, Isfahan, Iran.
2. Assistant Professor, Department of Microbiology, Islamic Azad University Isfahan (Khorasgan) Branch, Isfahan, Iran.
3. Dentist, Isfahan, Iran.
4. Assistant Professor, Department of Endodontics, Faculty of Dentistry, Shahrekord University of Medical Sciences, Shahrekord, Iran.

✉**Corresponding Author:** Parisa Ranjbarian, Faculty of Dentistry, Shahrekord University of Medical Sciences, Shahrekord, Iran.
Email: Parian_1381@yahoo.com **Tel:** +989132262014

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Abstract

Introduction: Debridement of root canal using appropriately safe and effective irrigants is the key factor for long-term success. Purpose of this study was to compare the antibacterial effect of propolis with 5.25% sodium hypochlorite, and 2% chlorhexidine against enterococcus faecalis.

Materials &Methods: In this study, 36 single-canal roots were used. The crown was removed and instrumentation was prepared by step-back technique, then teeth were sterilized and contaminated with *E.* Faecalis, and divided into four groups with 9 cases: group1: Propolis, group2: 5.25% sodium hypochlorite, group3: 2% chlorhexidine and group4: controls. Irrigants were injected by a 27-gauge syringe and roots were incubated in 37°C for one week. Sampling was done and inoculated to tryptone soy broth media, after 24 hours the turbidity was measured. Samples were also cultured on agar plates, and colony-forming units were counted as CFU/ml. Data were analysed using the Mann-Whitney test.

Results: The difference between propolis with mean value of 246.77 colonies and chlorhexidine with mean value of zero colonies, was significant (P=.002). Similarly, the difference between chlorhexidine and sodium hypochlorite with mean value 203.55 of colonies was significant and they had significant difference in turbidity (P=.002), too. No significant difference was observed between propolis and sodium hypochlorite with regard to the induced colonies (P=0.781) and their turbidity (P=0.495).

Conclusion: It can be concluded that antibacterial activity of 2% chlorhexidine against *E.* faecalis is more obvious than propolis or 5.25% sodium hypochlorite. But antibacterial activity of propolis over 5.25% sodium hypochlorite or vice versa was not confirmed.

Keywords: Enterococcus faecalis, Propolis,Sodium hypochlorite,Chlorhexidine,Root canal therapy

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مقایسه اثر آنتی باکتریال پروپولیس، هیپوکلریت سدیم ۵/۲۵٪ و کلرهگزیدین ۲٪ به عنوان داروی شستشوی داخل کانال بر ضد انتروکوکوس فکالیس: به صورت مطالعه آزمایشگاهی

مریم زارع جهرمی، آرزو طهمورث پور، نادیا همت، الهام مقدسی بروجنی، پریسا رنجبریان*

چکیده

مقدمه: دبریدمان موثر کانال ریشه به کمک داروهای شستشو، کلید موفقیت درمان ریشه می باشد. هدف از این مطالعه مقایسه اثرآنتی باکتریال پروپولیس با کلرهگزیدین ۲٪ وهیپوکلریت سدیم ۵/۲۵ ٪ بر ضد انتروکوکوس فکالیس است.

مواد و روش ها: در این مطالعه از۳۶ دندان تک کاناله استفاده شد. پس از قطع تاج، آماده سازی کانالها با استفاده از روش استپ بک انجام شد. سپس کانالها به باکتری انتروکوکوس فکالیس آلوده شدند. در مرحله بعد در شرایط آسپتیک، داروها به داخل کانال تزریق شدند. گروه۱: پروپولیس، گروه۲: هیپوکلریت سدیم۵/۲۵٪ ، گروه۳: کلرهگزیدین ۲٪ و۹ دندان هم به عنوان گروه کنترل در نظر گرفته شدند. نمونه ها به مدت یک هفته در دمای ۳۷ درجه سانتی گراد انکوبه شدند و نمونه گیری انجام گردید. نورسنجی توسط دستگاه اسپکتروفتومتر انجام گردید. نمونه ها همچنین بر روی پلیت کشت داده شدند و کلنی شماری انجام شد. داده ها با آزمون من ویتنی بررسی شدند.

یافته ها: پروپولیس با تعداد میانگین کلنی، ۲۴۶/۷۷ و کلرهگزیدین با عدم ایجاد کلنی، از نظر تعداد کلنی و کدورت تفاوت معنی داری دارند(P=.002) تفاوت تعداد کلنی و کدورت بین کلرهگزیدین و هیپوکلریت سدیم با تعداد میانگین کلنی ۲۰۳/۵۵، معنی دار است(P=0.05) . پروپولیس و هیپوکلریت سدیم از نظر کدورت (P=0.495) و تعداد کلنی (P=0.781)تفاوت معنی داری ندارند.

نتیجه گیری: می توان نتیجه گرفت که، کلرهگزیدین ۲٪ خصوصیت آنتی باکتریال بهتر علیه انتروکوکوس فکالیس نسبت به پروپولیس یا سدیم هیپوکلریت ۵/۲۵ ٪ دارد. اما برتر بودن خاصیت آنتی باکتریال پروپولیس و سدیم هیپوکلریت ۵/۲۵ ٪ نسبت به هم ثابت نشد.

واژگان کلیدی: انتروکوکوس فکالیس، پروپولیس، هیپوکلریت سدیم، کلرهگزیدین، درمان کانال ریشه

Introduction

The pulp chambers and root canals of necrotic teeth are filled with gelatinous masses of pulp remnants and tissue fluid. [1, 2, 3] The success of endodontic therapy depends on removal of necrotic pulp debris and microorganisms from the root canal. Residual microorganisms in pulpal spaces and dentin tubules may cause persistent infection after endodontic therapy. [4]One of the most important microorganisms in endodontic is Enterococcus faecalis which has the ability to penetrate into the dentinal tubules and survive in root canals without other bacterial support [5] and been frequently isolated from infected pulp and persistent infections in post-endodontic treatment.[6] Although canal instrumentation is a basic technique for debridement of the root canals, at the same time, irrigating solutions are being used to disinfect the root canal system.[7] Several irrigating solutions are being used in today's modern practices. Sodium hypochlorite is the most common irrigating solution that has antimicrobial activity as well as lubricating and ability of tissue solving.[8] Unfortunately, hypochlorite has several disadvantages such as metal corrosion, irritating to skin and eyes, strong odor [3], it can also elicit severe inflammatory reactions on the periapical tissues [9]at high concentration [10] 2% Chlorhexidine solution is a cationic detergent which is compatible with the periapical tissues [11], mainly applied in endodontics as an irrigating solution [12, 13], a broad-spectrum antimicrobial agent that has substantive antibacterial activity and relatively low toxic effects [14], but it does not present tissue dissolving activity. [15]

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Despite of the efforts to introduce an appropriate canal irrigating solution, it was unsuccessful. Another irrigating solution presented in endodontics is propolis known in traditional medicine. [16, 17] Propolis is a sticky, resinous material gathered by bees from herbal buds and mixed with secreted beeswax [3] and it is rich in flavonoids as its biologically active component. [1, 18] Its ethanolic extract has different biological properties such as: antibacterial, antifungal, antiviral, anti-inflammatory, local anesthetic, antioxidant, and cytostatic properties. [19] Recently, propolis is applied as an intracanal medication and can be considered as the drug of choice for the canal irrigation solution. [20]

Since the herbal medication has advantages such as minimal side effects, better tolarance by patients and renewed by nature over conventional endodontic irrigation, [21] the aim of this study was to compare the antibacterial effect of propolis canal irrigating solution with sodium hypochlorite 5.25%, and 2% chlorhexidine against enterococcus faecalis.

Materials & Methods

This ex-vivo study was performed in the Faculty of Dentistry of Islamic Azad University, Isfahan Branch (Khorasgan); Isfahan, Iran in 2015.

A total of 36 extracted human single-rooted teeth without crack or pulp calcification were used. To remove surface debris each tooth was immersed in a sodium hypochlorite 5.25% (CERKAMED, Poland) for 20 minutes and stored in saline (Iranian Parental and Pharmaceutical Products Co., Iran) prior to use, then the radiographs were used to rule out roots with calcification or sever root curvature. The crown portion was removed at CEJ and the length of instrumentation was standardized at 15 mm. Instrumentation was conducted by widening the coronal part with Gates Glidden from size 2 to size 4 (Mani, Japan) and then the apical portion was prepared by step-back technique using K-type files (Mani, Japan) until apical foramen match size 30 after that each root apex conditioned with phosphoric acid 10% (Kiristalin, Germany) for 30 seconds and next the primer (Kiristalin, Germany) was applied on root surface and after 30 seconds bonding agent (Kiristalin, Germany) was added to the primer and gently spread on the root surface then light cured for 30 seconds and composite flow with 1mm thickness (Kiristalin, Germany) was next applied and cured for 40 seconds to completely seal apex.[10]

In the next step, to remove smear layer, the canals were irrigated with 10 cc Sodium hypochlorite 5.25% and then 10cc EDTA 17% (Merk, Germany) each for 4 minutes and after that 10cc saline was used and teeth were wrapped in aluminum foil and sterilized twice with autoclave (Iran Tolid Medical Industries Co, Iran) at 121 ° C and pressure of 15 pounds per square inch for 30 minutes. To check the accuracy of canals' sterility, four of them were randomly chosen and sampled with paper point (Aria dent, Iran), then transferred to sterile broth and agar medium and incubated for 1 week at 37 ° C. After one-week incubation, medium showed neither turbidity nor any sign of colony growth, indicating the sterility of the samples. After ensuring the sterility of samples in anaerobic conditions, an overnight bacterial culture of *E* .faecalis (ATCC 29212) in brain heart infusion(BHI) at concentration of 0.5 Mc Farland (1.5 ×108 CFU) was added into the canals by a sterilized sampler , to enhance the growth of E. faecalis, broth culture was also added. Once every 3 days, microbial samples were prepared according to McFarland turbidity standard No. 0.5 and injected into the canal, after one week, the samples were irrigated with 10cc saline and divided into 4 groups with sample size of 9 for each group; group 1 was exposed to 40ml sodium hypochlorite 5.25% , group 2 was exposed to 40ml chlorhexidine 2%, group 3 was exposed to 40ml of 11% alcoholic extract of propolis (Agriculture and natural resources research center, Isfahan, Iran) prepared by diluting 30% alcoholic extract with saline in 2:1 ratio[22] and group 4 as a control group.

Concentration of 5.25% sodium hypochlorite was chosen because it is one of the most commonly used concentration for endodontic research. [21]

Irrigants were injected by a 27-gauge insulin syringe. The syringe was held in the root canal center with special care without touching the walls and bottom of roots canal. Excess of irrigants removed by a suction tube. Then, the canal orifice was sealed with 3mm temporary restoration (zonalin (Kemdent, England)) and covered with two layers of nail polish and all samples were incubated for 1 week at 37 ° C, after accessing the canals, they were irrigated with 10cc saline. Canal sampling was done with paper points in aseptic conditions. Samples were inoculated to tryptone soy broth (TSB) media in test tubes, after 24 hours of incubation, the turbidity was measured by a spectrophotometer at 540 nm.

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In order to enumerate the colony forming units, a dilution series of each sample was prepared with phosphate-buffered saline. A convenient inoculum volume (100 µl), in terms of spreading, absorption, and calculations, was transferred to mitis salivarius agar plates. For bacterial culture in Mitis salivarius agar medium, the diffusion method was used because large colonies are created in this way and it is easier to count, and then, the plates were incubated for 24 hours.

After the incubation period, the numbers of plates' CFUs/ml were calculated. The average number of CFU/ml of each group was analyzed using Mann-Whitney nonparametric test, statistical significance was considered $P < 0.05$.

Results

Distributions of colonies in four groups are presented in table 1**.** There were significant differences (*P*=0.003) between control group and sodium hypochlorite, also between control group and chlorhexidine either in the number of colonies or in turbidity(*P*=0.003). The number of colonies and turbidity had significance difference in propolis compared to control groups (*P*=.001 and *P*=.002, respectively) (Table1).

The number of colonies of propolis and chlorhexidine indicated the significant difference (*P*=0.002). Turbidity of propolis and chlorhexidine samples represented the significant difference (*P*=0.002), too. Propolis and sodium hypochlorite did not illustrate the significant differences either in the number of colonies (*P*=0.781) or in the turbidity (*P*=0.495). Chlorhexidine and sodium hypochlorite showed significant differences (*P*=0.05) either in the number of colonies or in turbidity.

The results of the current study indicated that compared to the control group, the reduction of 63.66% in the number of colonies was occurred in the presence of sodium hypochlorite, chlorhexidine showed 100% reduction in numbers of colonies and propolis caused 59.5% reduction in colonies count.

Table 1. Distribution of colonies in four groups

Group	teeth	Mean number of colonies	Comparison of mean number of colonies in four groups p-value	Turbidity	Comparison of turbidity in four groups p-value
Propolis	9	246.77	0.0001	0.685	0.0001
Chlorhexidine	9	0		0	
sodium hypochlorite	9	203.55		0.513	
Control	9	610		1.735	

Discussion

In present study, 2% chlorhexidine compared to propolis and 5.25% sodium hypochlorite showed the most antibacterial properties against E. faecalis. But, propolis antibacterial properties over sodium hypochlorite or vice versa were not confirmed.

Clinical studies have shown that anaerobic bacteria play the major role in pulp and periapical diseases. [23, 24] Due to canal anatomic variation, purely mechanical preparation cannot thoroughly clean the root canal space. [25] The results suggested that chlorhexidine than sodium hypochlorite and propolis had significantly higher antibacterial activity.

In general, wide varieties of studies in different situation were taken place to consider the antibacterial effect of endodontic irrigating solutions especially against E. faecalis. [3, 14, 15, 26] In 2015, Saxena et al. [21] conducted a study on in vitro evaluation of antimicrobial activity of propolis as herbal extracts and compared its activity with 2.5% sodium hypochlorite against Enterococcus faecalis. They explained that 2.5% sodium hypochloritehad had higher zone of inhibitation. The result of this study differs from that of the present study. This difference may be due to the difference of the used method in both studies. In Saxena et al.'s study[21], they placed sodium hypochlorite and propolis as discs in the culture plates but in the present study, sodium hypochlorite and propolis were used as intracanal irrigants injected to root canals infected with E. faecalis.

However, in similar study, in 2014, Garg et al. [27] evaluated the antimicrobial efficacy of propolis with 5.25% sodium hypochlorite and represented no statistically significant difference. Also, in a study

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conducted by Qathami et al. [3], in 2003, colony counts showed that the antimicrobial activity of propolis and sodium hypochlorite was equivalent, which is consistent with the present study of approximately equal antibacterial properties of propolis and sodium hypochlorite 5.25%.

Antimicrobial activity of sodium hypochlorite is due to the release of chlorine ions which deactivate the bacterial sulfhydryl enzymes and nucleic acids, and denature the microorganisms protein. [28] Another common antimicrobial solution for canal irrigation is chlorhexidine. Chlorhexidine is a cationic Guanine base and a broad-spectrum disinfectant against gram-positive and-negative anaerobic bacteria, fungi, yeasts, and some viruses such as hepatitis and AIDS [29], but it doesn't have ability to solve tissue. [14]

Carbajal Mejía et al [30] in 2014 compared the effect of propolis and chlorhexidine against Enterococcus faecalis, and concluded that there was no difference between them. The result of this study differs from the result of the present study. In their study, the propolis and chlorhexidine were used as an intracanal medicament for 14 days, but in the current study, propolis and chlorhexidine were used as intracanal irrigants and this could be related to the difference of results between the mentioned study and the present study. In 2010, Kandaswamy et al. [31] investigated the dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morindacitrifolia juice, 2% povidone iodine and calcium hydroxide, and among all, 2% chlorhexidine gel was the most effective against enterococcus faecalis. In the related study accomplished by Ferraz et al., [32] 2% chlorhexidine gel than any concentration of sodium hypochlorite had more antibacterial efficacy. The results of these two studies based on greater impact of chlorhexidine, support the result of the present study.

The need to employ the natural material without disadvantageous side effects, on the one hand, and with minimal tissue irritation and the most antibacterial effect, on the other hand, lead to the introduction of new materials such as propolis. Propolis is a resinous complex mixture of chemical components. [33] Propolis may act against a wide range of bacteria, fungi, yeasts, viruses and invading larvae. [34] In several studies, antibacterial activity of propolis has been reported in different ways. It was shown that propolis inhibited the bacterial growth by preventing cell division, disorganizing the cytoplasm, the cytoplasmic membrane and the cell wall, caused a partial bacteriolysis, and inhibited protein synthesis. [35, 36] Among propolis constituents, flavonoids had the most effect. [26, 35, 36] Antibacterial properties of propolis can be attributed to the suppression of virulence factor coagulase, reduction of lipase and prevention of biofilm formation [37] and, in this way, it has relatively good antibacterial properties compared to sodium hypochlorite.

Of course, every study has its own specific limitation that will impair the result. In present study, viable but not cultivable colonies could be misinterpreted by the used method, or if this study performed as a clinical trial, the results can be more reliable.

Conclusion

Within the limitation of this study, it can be concluded that 2% chlorhexidine compared to propolis and 5.25% sodium hypochlorite had the most antibacterial activity against E. faecalis. Nevertheless, propolis antibacterial properties over sodium hypochlorite or vice versa were not confirmed. Since the studied irrigants had potential bacterial activity against E. faecalis, they all can be consider to be used in root canal treatment but chlorhexidine may be the material of choice.

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Conflict of interest: We declare no conflict of interest.

Authors' Contributions

In this study, Maryam Zare Jahromi was superviser, Arezoo Tahmourespour was adviser and Nadia Hemmat, Elham Moghadasi Broujeni and Parisa Ranjbarian were partners of project**.**

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