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Original Article

Antibacterial efficacy of lavandula officinalis extract, sodium hypochlorite and chlorhexidine gluconate solutions as root canal irrigations: A comparative analysis

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Abstract

Introduction: This in vitro study aimed to compare the antimicrobial effect of lavandula -0fficinalis extract, with sodium hypochlorite (NaOCL) and chlorhexidine gluconate (CHX), as root canal irrigants, on Enterococcus faecalis (EF).

Materials &Methods: Seventy five extracted single-rooted premolars were selected. Root canals were prepared using rotary ProTaper system and were infected with the culture of E. faecalis. Specimens were randomly divided into five groups (n = 15), Group 1, 2: lavandula extracts (0.26 and 0.52 mg/mL), Group 3: 2.5%NaOCL, Group 4: 2%CHX, Group 5: Normal Saline. Irrigation was performed for each group for 5, 10 and 15 min. The viable bacteria obtained by collecting the canal dentin chips. Data analysis was performed with Kruskal-Wallis and Mann-Whitney u tests.

Results: The mean number of viable bacteria was significantly reduced after 5 min exposure to lavandula solutions ($p<0.05$). A significant difference also existed between different times in the NaOCL group, being significant between 5 and 15 min ($p<0.05$), but there was no significant difference between different times in the CHX group. Comparison of the mean number of viable bacteria between different groups at different exposure times revealed that the difference between lavandula and NaOCL solutions with CHX was significant at 5 and 10 min ($p<0.05$), however, no statistically significant difference was observed between lavandula solutions and NaOCL.

Conclusion: lavandula extract was effective in killing of EF. Further studies are necessary to fully understand its other properties such as tissue solubility, removal of smear layer and impact on dentin structure.

Keywords: Chlorhexidine gluconate, Enterococcus faecalis, Lavandula, Root canal irrigants, Sodium hypochlorite

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مقایسه تاثیر ضد میکروبی عصاره اسطوقدوس و محلولهای هیپوکلریت سدیم و کلرهگزیدین گلوکونات به عنوان شستشو دهنده های کانال ریشه

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چکیده

مقدمه: هدف از این مطالعه آزمایشگاهی مقایسه اثر ضد میکروبی عصاره اسطوقدوس به عنوان یک شستشو دهنده کانال ریشه با هیپوکلریت سدیم ۲.۵٪ و کلرهگزیدین گلوکونات ۲٪ در مقابل انتروکوکوس فکالیس بود.

مواد و روش ها: ۷۵ پره مولر تک ریشه کشیده شده انتخاب شدند. کانالهای ریشه ها با استفاده از سیستم روتاری پروتیپر آماده سازی شده و با کشت انتروکوکوس فکالیس عفونی شدند. نمونه ها بطور تصادفی به ۵ گروه (۱۵ تایی)، گروه ۱و۲: اسطوقدوس (0.26 mg/mLو 0.52)، گروه ۳: هیپوکلریت سدیم ۲/۵ درصد، گروه ۴ (کلرهگزیدین گلوکونات ۲ درصد) وگروه ۵: نرمال سالین تقسیم شدند. شستشو برای هر گروه به مدت ۵، ۱۰ و ۱۵ دقیقه انجام شد. باکتریهای زنده بدست آمده از دنتین چیپس کانال ریشه محاسبه گردیدند. آنالیز داده ها با استفاده از آزمونKruskal-Wallis و Mann-Whitney u انجام شد.

یافته ها: میانگین تعداد باکتریهای زنده پس از ۵ دقیقه مواجهه با محلولهای اسطوقدوس بطور معنی داری کاهش یافت ($p<0.05$). تفاوت معنی داری بین زمانهای مختلف در گروه هیپوکلریت سدیم ملاحظه گردید که این تفاوت بین ۵ و ۱۵ دقیقه معنی دار بود ($p<0.05$)، اما بین زمانهای مختلف در گروه کلرهگزیدین گلوکونات تفاوت معنی داری وجود نداشت. مقایسه میانگین تعداد باکتریهای زنده بین گروههای مختلف در زمانهای متفاوت حاکی از تفاوت معنی دار محلولهای اسطوقدوس و هیپوکلریت سدیم پس از ۵ و ۱۰ دقیقه بود ($p< 0.05$). اختلاف آماری معنی داری بین محلولهای اسطوقدوس و هیپوکلریت سدیم با اسطوقدوس وجود نداشت.

نتیجه گیری: عصاره اسطوقدوس در کشتن انتروکوکوس فکالیس موثر است. مطالعات بیشتر جهت بررسی سایر ویژگیهای این عصاره از جمله حلالیت بافتی، حذف اسمیر لایرو تاثیرآن بر ساختار دنتین پیشنهاد می شود.

واژگان کلیدی: کلرهگزیدین گلوکونات، انتروکوکوس فکالیس، اسطوقدوس، شستشو دهنده های کانال ریشه، هیپوکلریت سدیم

Introduction

Studies have demonstrated the role of bacteria and their products as the major factor in development of the pulp and periapical diseases.[1-3] Enterococcus faecalis is the most frequently observed bacteria in the root canal. The ability of this gram-positive anaerobic bacterium to penetrate the dentinal tubules as well as to form biofilm and tolerate unfavorable environmental conditions can justify its relatively high prevalence.[2,3] Mechanical preparation of the root canal is the first stage of canal cleaning; accordingly, supplementary irrigation solutions are necessary and important for treatment.[4] The selection of irrigants is very important due to their different functions in removal of debris, smear layer, and bacteria from the root canal system. Different chemical formulations of irrigants exert varying effects on pulp, necrotic tissues, and microorganisms.[5] Sodium hypochlorite became a widely used irrigant after its introduction by Walker.[6] Almost all studies have mentioned its appropriate antibacterial effects.[4,6] Although this solution is the irrigant of choice in the treatment of necrotic teeth due to its antimicrobial activity and unique tissue solubility properties, it is toxic to living tissue and its extrusion from the apex is associated with pain and emphysema. Moreover, it is allergen, its taste is unpleasant for patients, and its vapor is eye-irritant.[3,5,6] Therefore, searching for a solution with fewer side effects and similar or better antimicrobial properties is still ongoing. 2% CHX is another irrigant, which has been supported as an ultimate cleaning solution, especially in endodontic retreatments, due to its long-term stability resulting from binding to hydroxyapatite.[2] Tooth discoloration and some other side effects such as loss of sense of taste, irritation of oral mucosa, dry mouth and tongue

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discoloration limit its use as a long term irrigant. It has been also found that the cleaning effect of CHX in the walls of the root canal is lower than NaOCL.[5] MTAD (a mixture of tetracycline, citric acid, and detergent) was an attempt to achieve a better irrigant, but according to the in vitro studies, its bactericidal efficacy was reported less than sodium hypochlorite.[3]

Given the ever-increasing number of antibiotic-resistant species due to misuse of antibiotics and complications of synthetic irrigants (such as immune suppression, hypersensitivity, allergic reactions), as well as the phytotherapeutic potential of herbal irrigant solutions,[3,7-9] researchers have recently begun to investigate herbal alternatives. many studies have found that herbal agents such as green tea, Triphala, Monrinda Citrifolia, Zataria Multiflora Boiss, Satureja Khuzestanica Jamzad, Carvacrol, and Arctium Lappa A. nilotica, A. barbadensis, C. sylvestris, A. sativum, M. recutitia L, C. sinensis, C. limonum, propolis, P. guajava, P. corylifolia, R. lancia, S. persica, S. aromaticum, M. alternifolia, C. longa, G. glabra can be used as an alternative intra-canal medicament and can be used as potential root canal irrigants because of their anti-inflammatory, antimicrobial and immune-modulating activity.[3,5,6,8-12]

Lavandula is a plant with many medicinal properties, which has been used since ancient times. Essential oil of this species has been used in the perfume and cosmetics industry from hundreds of years ago. It is also used as an essential oil in drinks, ice cream, candy, and gum. Many studies enumerated interesting chemical and biological properties of this plant, including sedative, spasmolytic, anti-inflammatory, antioxidative, hypolipidemic, antiviral, antifungal, and antibacterial effects.[7,12] It is proven that the oil extract of lavandula is effective in treatment of infections caused by antibiotic-resistant bacteria.[7,12]

Benbelaid et al. evaluated the antimicrobial activity of Lavandula multifida oil extract on gram-positive and gram-negative pathogens including Enterococcus faecalis, using impregnated discs. Their results showed that the extract has a tremendous impact on Enterococcus faecalis.[7]

In an in vitro study, using agar diffusion and broth dilution methods, Blazekovic et al. evaluated the effect of ethanolic extract of lavandula on 31 species of bacteria, fungi, dermatophytes, and molds and found that, as the most abundant ingredients, Linalool and Linalylacetate are responsible for antibacterial and antifungal effects of this plant. These researchers suggested this herbal compound as an acceptable alternative for NaOCL.[13]

In the study of Imelouane et al. the dental antimicrobial activity of lavandula essential oil has been demonstrated on 22 bacterial species except Pseudomonas aeruginosa. In this study, the essential oil of lavandula was introduced as a food preservative. This compound improves health and (canned) food survival through eliminating or reducing pathogens which grow on food.[14]

The aim of this experimental study was to compare the antibacterial effect of Lavandula extracts as a root canal irrigant with 2.5% NaOCL and 2% CHX on Enterococcus faecalis (E. Faecalis).

Materials&Methods

Sample preparation: In this in vitro experimental study, 75 single canal maxillary and mandibular bicuspids were stored in normal saline to prevent dehydration. Number of the canals was verified by periapical radiographs and samples were visually inspected for existence of defects such as external resorption, crack and caries. All teeth had a single root canal and root lengths between 11-14 mm. The crown was removed at the cementoenamel junction with a high speed diamond fissure bur (Tizkavan, Tehran, Iran). Working lengths were determined with K-file #10 (Dentsply, Switzerland). The root canals were then instrumented using the crown-down technique and rotary instruments (ProTaper, Dentsply Maillefer, Ballaigues, Switzerland), and the canals were enlarged to an apical size of F3. At all stages of cleaning and shaping of the canals normal saline was used as an irrigant. To remove the smear layer 10ml Ethylene diaminetetraacetic acid (EDTA) 17% was used for 1 minute followed by 5ml NaOCl 5.25%. To prevent apical leakage the root apex was sealed with resin composite. The Cryo Tube containing the teeth and BHIB (Brain Heart infusion broth) medium were sterilized in autoclave for 20 minutes at 121˚c.

Phytochemical preparation: Extraction was performed through maceration method. 15 g dried lavandula officinalis (which was provided from the Research institute of forests rangelands of Iran) was poured into 150 mL water and mixed by magnetic stirrer for 24 hours, after that it was filtered with a filter paper. The filtrate solution was centrifuged for 20 minutes at

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12,000 rpm. The supernatant was removed and poured into the perfect clean plate glass and dried for 3-4 days thoroughly under the hood. After drying, the powder was used for testing.

To determine the minimum inhibitory concentrations of each irrigant, the agar dilution method was used. Specific concentration of each irrigant was added to test tubes, containing 20 ml of nutrient agar medium, which was then poured in7 cm in diameter circles, into a disposable plate. The concentrations used for each chemical were 1 to 50 ppm. Agar plates were placed at 40 ° C for 30 minutes to dry out the surface moisture. Then 10 µL of bacteria was placed on the agar. The plates were incubated at the specified temperature (37°C) for 24h .The lowest concentration of a chemical substance that can inhibit the growth of microbes is called the minimum inhibitory concentration (MIC). MIC of the materials for E.faecalis was then examined. As a positive control, a commercial standard antibiotic was used (amoxicillin and nalidixic acid for E. Faecalis).

Antibacterial evaluation: A pure culture of E. Faecalis (Iranian Type Culture Collection [ATCC] 29212) (Pasteur Institute, Tehran, Iran) was grown on Mueller-Hinton agar (Merck Co, Germany), inoculated into Mueller Hinton broth (Merck Co, Germany), incubated at 37°C overnight and adjusted to an optical density (OD600) of 1 with sterile Mueller-Hinton broth.

Irrigation procedure: The prepared teeth (n=75) were randomly divided into 5 groups (n=15) and each group was divided into three subgroups. These groups included lavender 0.26 mg/ml and 0.52 mg/ml, 2.5% sodium hypochlorite (Sehat, Tehran, Iran), 2% chlorhexidine gluconate (clorhexidina s, Dentscare LTDA, brasil) and normal saline. Under aseptic conditions, the teeth were removed from Cryo Tubes and root canals were dried with sterile paper points #30 (Panadent, China) and were filled with bacterial suspensions with a sterile insulin syringe. Teeth were then transferred to Cryo Tubes containing BHI medium and were incubated for 48 h at 35 ° C under micro aerophilic condition (MART system 5% CO2, 5.9% O2, 7.2% H2, 79% N2). After incubation, the teeth were removed from the Cryo Tube and their canals were rinsed three times with 10 mL normal saline with an insulin syringe and dried with sterile paper points # 30. Then, each canal was filled with irrigant, using an insulin syringe. After 5,10 and 15 min, the canals (5 canals in each group) were dried with paper points # 30, and to inactivate the irrigants, the canals were filled with Soy-Lecithin-Poly-Sorbate 20 (Merck Co, Germany), which was finally removed with sterile paper points # 30. To determine the viable bacterial count, by Hedstrom file #40 (H file, Dentsply, Swiss) 0.1 gram dentin chips were collected from the canal and mixed with 10 ml of normal saline containing Tween 80 (Merck Co, Germany) 1% in mixture, and shaken heavily for 30 seconds, until bacteria was separated. For counting viable bacteria, 1 ml of the mentioned suspension was cultured on Caso agar (Soybean Casein digest Broth, Casein-peptone Soymeal-peptone Broth) medium. After 48 h incubation at 35°C, the rate of bacterial colonies was counted and reported in CFU/ dentin chips form. Kruskal-Wallis and Mann-Whitney u tests were used to evaluate the differences in the mean cell viability values of the experimental solutions. A level of $p<0.05$ was statistically accepted as significant.

Results

Table 1 compares the mean number of viable bacteria in different groups at three irrigation times. Accordingly, there was a significant difference in the number of viable bacteria between the groups at 5 and 10 min irrigation times but the difference was not statistically significant in 15 min.

Table 1. Mean number of viable bacteria in different times of exposure to irrigant solutions (L_1= lavandula 0.26 mg/ml, L_2 = lavandula 0.52 mg/ml)

Time (min)	Irrigant	Mean	Pvalue
5	L_1	28.00±31.44	0.34
	L_2	28.67±24.6	
	NaOCL	18.00±19.35	
	CHX	52.00±43.02*	
10	L_1	11.67±16.22	0.47
	L_2	15.60±16.49	
	NaOCL	10.00±13.50	
	CHX	30.78±23.41*	
15	L_1	5.00±9.06	0.55
	L_2	7.67±9.98	
	NaOCL	4.00±8.90	
	CHX	13.00±24.77	

In 5 min irrigation, Mann-Whitney u test showed that the mean number of viable bacteria in lavandula

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0.26 mg/mL and 0.52 mg/mL and NaOCL irrigant groups was similar, however, the mean number of viable bacteria in the CHX group was significantly greater than the other three ($p<0.05$). Thus, the difference between lavandula and NaOCL solutions and CHX was significant ($p<0.05$), but no significant difference was found between lavandula solutions and NaOCL (Table 2). The Mann-Whitney u test for 10 minutes irrigation showed that CHX had significantly the lowest antibacterial effect among 4 irrigation solutions ($p<0.05$). Other irrigants had no significant difference with each other (Table 2).

Table 2. Comparison of viable bacteria count in 5, 10 min exposure to irrigants

Irrigant (I)	Irrigant (J)	P value	
		5 min	10 min
L_1	L_2	0.806	0.486
L_1	NaOCL	0.486	0.967
L_1	CHX	0.044	0.026
L_2	NaOCL	0.435	0.512
L_2	CHX	0.036	0.044
NaOCL	CHX	0.014	0.2

Discussion

This in vitro study evaluated the antibacterial effect of lavandula, as a root canal irrigant, on E. faecalis. It is the most common bacteria which is frequently observed in root canal treated teeth.[2] Therefore, E. faecalis was used in this study to determine the antimicrobial efficacy of irrigant solutions. The results of this study indicated that the antibacterial effect of lavandula extract was not significantly different with 2.5% NaOCL after 5 min, but this irrigant significantly reduced the mean number of E. faecalis compared with 2% CHX. The antibacterial activity of this plant is in line with results of other researchers. Dadalioglu et al., Lis-balachin et al and Goren et al. have demonstrated the powerful antifungal and antibacterial ability of different species of lavandula.[15-17] Numerous researchers have studied various species of lavandula and stated that 1,8-Cineole, Linalool, linaly acetate, β-phellandrene, and Fenchone are found in most of these species. Significant differences exist between major constituents of this plant, which are attributed to climatic, seasonal, geographical conditions, techniques of extractions, the period of harvest and the duration of drying as well as genetic differences of the species.[14,18] Antimicrobial activity of the plant is caused by synergistic effects of a range of major and minor constituents. Sikkema et al. investigated the function of monoterpens as its major constituents and stated that they exert their antimicrobial effects through diffusion and structural damage to cell membranes.[19] Griffen et al. claimed that the antimicrobial activity of terpenoids depends on several factors including hydrogen bonding, water solubility, and molecular size.[20] Importantly, in comparison with the cited studies,[15-17] this study was carried out to evaluate the antimicrobial effects of this plant in the presence of dentin and in the root canal system. It is proven that the antibacterial effects of irrigants such as NaOCL, CHX and iodide potassium iodine are reduced in the presence of dentin, i.e. dentin has an inhibitory effect on the activity of these irrigants.[2,4] Therefore, in this study, we reconstructed the root canal system under similar conditions and evaluated the antibacterial ability of this plant extract as an irrigant.

As an irrigant, sodium hypochlorite is widely used in endodontic treatment. Interestingly, no significant difference was found in this study between lavandula and 2.5% NaOCL in 5 minutes. NaOCL is highly caustic and as a nonspecific agent, its action is not limited to necrotic tissues [21] and has adverse effects on dentin including reduced modulus elasticity and flexural strength.[3,22,23] Therefore, seeking for a solution with similar or better antimicrobial properties and fewer side effects is ongoing and in this regard, our study aimed to find a herbal irrigant.

Reduced bacterial content in the negative control group was due to washing out of the microorganisms with normal saline and it is noteworthy that saline does not have any antibacterial effect. During this research, smear layer was removed using 17% EDTA and 5.25% NaOCL after bio-mechanical preparation of the root canal, and then the bacteria were cultured. This procedure was performed in all groups, thus, bacteria and irrigants had similar opportunity to enter the dentinal tubules and contact with dentin.

Owing to the reserves of biologically active substances, plants have been considered from long times ago. Besides several therapeutic properties, lavandula is an antioxidant and highly tissue compatible plant which is probably safe for the periapical tissues. Given the anti-inflammatory and analgesic properties of this plant compared with conventional irrigants of root canal, it may have additional beneficial effects.[24,25] It is proven

that the oil extract of this plant is effective in treatment of infections caused by antibiotic-resistant bacteria.[7] Easy access, cost-effectiveness and lack of microbial resistance pose it as an alternative irrigant. Considering the complexity of the root canal system and the importance of necrotic pulp remnants removal from regions not in access of instruments, it is proposed to research on lavandula extract's tissue solubility. For future studies, evaluating the impact of the extract on sealing ability of root canal filling materials, as well as its effect on organic and inorganic structure of dentin and removal of smear layer are suggested.

Conclusion: within the limitations of this invitro study, the efficacy of lavandula extract was similar to NaOCL and was better than CHX. The use of Lavandula as an endodontic irrigant might be advantageous because it is a biocompatible antioxidant[7] and not likely to cause the severe injuries to patients that might occur through NaOCL accidents. More research is needed to include it in irrigation protocol of root canal treatment.

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Conflict of interest: We declare that there is no conflict of interest.

Authors' Contributions

The study was designed by Narges Farhad mollashahi. Data were collected by Pouya Mehmandoust, Ali Ghasemi. Analysis and interpretation of data, drafting of the manuscript and critical revision of the manuscript for important intellectual content were pre-formed by Narges Farhad mollashahi. Supervision of the study was performed by Narges Farhad mollashahi.

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