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

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Received: 23 Aug 2015 Accepted: 28 Feb 2016

Abstract

Introduction: This in vitro study aimed to compare the antimicrobial effect of lavandula - officinalis 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 groups 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 کلرگزیدن گلوکان‌ها به عضوبخش دهنده‌های کانال ریشه

یوسف مهمندودست، نرگس فرده ملاشاهی، علی قاسمی

چکیده
مقدمه: هدف از این مطالعه آزمایشگاهی مقایسه اثر ضد میکروبی عصاره اسطوقدوس به عضوبخش دهنده‌های کانال ریشه با هیپوکلریت سدیم و کلرگزیدن گلوکان‌ها بود.

مواد و روش ها: 75 پره مول تک ریشه کسب شده از شرکت تلیف از سیستم روتاری پوستک آماده بود. سازی پهلوی و با کشت انتهای‌کوس‌های فکالس‌های عضوبخش دهنده‌های کانال ریشه به پراپانول (15 تا) و هیپوکلریت سدیم 0.05 درصدی و کلرگزیدن گلوکان‌ها 2 درصدی و گلوکان‌های 2 درصدی و گلوکان‌های 0.52 درصدی، گلوکان‌های 0.26 درصدی، گلوکان‌های 0.52 درصدی، گلوکان‌های 0.26 درصدی و گلوکان‌های 0.52 درصدی را برای 15 دقیقه تیز کردند. بهترین نتیجه در 5 دقیقه، 10 دقیقه و 15 دقیقه انجام شد. باکتری‌های زند به استفاده از دنبی خیس کانال ریشه محاسبه گردید. آنالیز داده‌ها با استفاده از آزمون Mann-Whitney و آزمون Kruskal-Wallis انجام شد.

نتایج: با استفاده از گروه‌های زند به مدت 5 دقیقه، 10 دقیقه و 15 دقیقه و توجه به نتایج آزمون Mann-Whitney و آزمون Kruskal-Wallis نتایج بهتری بدست آمد. نتایج نشان می‌دهد که با کمک این ماده‌ها، بهترین نتیج بهتری بدست آمده است. بهترین نتایج در گروه 2% CHX و گروه 0.52 درصدی کلرگزیدن گلوکان‌ها بدست آمد.

واژگان کلیدی: کلرگزیدن گلوکان‌ها، انتهای‌کوس‌های فکالس‌های اسطوقدوس، شستشو به عنوان دهنده‌های 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. 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.

Mechanical preparation of the root canal is the first stage of canal cleaning; accordingly, supplementary irrigation solutions are necessary and important for treatment.

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.

Sodium hypochlorite became a widely used irrigant after its introduction by Walker. Almost all studies have mentioned its appropriate antibacterial effects. 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.

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. Tooth discoloration and some other side effects such as loss of sense of taste, irritation of oral mucosa, dry mouth and tongue...
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.\cite{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.\cite{13}

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,\cite{3,5,6,8,9} 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. recutita 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.\cite{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, spasmylytic, anti-inflammatory, antioxidative, hypolipidemic, antiviral, antifungal, and antibacterial effects.\cite{7,12} It is proven that the oil extract of lavandula is effective in treatment of infections caused by antibiotic-resistant bacteria.\cite{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.\cite{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.\cite{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.\cite{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
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 in 7 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 24 h. 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 agar (Soybean Casein peptone 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 forms. 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 (L1 = lavandula 0.26 mg/ml, L2 = lavandula 0.52 mg/ml)**

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Irritant</th>
<th>Mean ± SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>L1</td>
<td>28.00±31.44</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>28.67±24.6</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>NaOCL</td>
<td>18.00±19.35</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHX</td>
<td>52.00±43.02</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>L1</td>
<td>11.67±16.22</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>15.60±16.49</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaOCL</td>
<td>10.00±13.50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHX</td>
<td>30.78±23.41</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>L1</td>
<td>5.00±9.06</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>7.67±9.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NaOCL</td>
<td>4.00±8.90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CHX</td>
<td>13.00±24.77</td>
<td></td>
</tr>
</tbody>
</table>

In 5 min irrigation, Mann-Whitney u test showed that the mean number of viable bacteria in lavandula
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 monoterpenes as its major constituents and stated that they exert their antimicrobial effects through diffusion and structural damage to cell membranes. Griffith et al. claimed that the antimicrobial activity of terpenoids depends on several factors including hydrogen bonding, water solubility, and molecular size. Importantly, in comparison with the cited studies, 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.

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 and has adverse effects on dentin including reduced modulus elasticity and flexural strength. 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.

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

<table>
<thead>
<tr>
<th>Irrigant (I)</th>
<th>Irrigant (J)</th>
<th>P value 5 min</th>
<th>P value 10 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>L₁</td>
<td>L₁</td>
<td>0.806</td>
<td>0.486</td>
</tr>
<tr>
<td>L₁</td>
<td>NaOCL</td>
<td>0.486</td>
<td>0.967</td>
</tr>
<tr>
<td>L₁</td>
<td>CHX</td>
<td>0.044</td>
<td>0.026</td>
</tr>
<tr>
<td>L₂</td>
<td>NaOCL</td>
<td>0.435</td>
<td>0.512</td>
</tr>
<tr>
<td>L₂</td>
<td>CHX</td>
<td>0.036</td>
<td>0.044</td>
</tr>
<tr>
<td>NaOCL</td>
<td>CHX</td>
<td>0.014</td>
<td>0.2</td>
</tr>
</tbody>
</table>

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. 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. 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.
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.

Acknowledgments
We thank the Vice Chancellor for Research of Medical Sciences University of Zahedan for approving and financial support of this study.

Funding: This study was a part of thesis and research project (Grant No: 6106) supported and funded by Zahedan University of Medical Sciences.

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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lavandula as a herbal root canal irrigant