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Comparative evaluation of the antimicrobial efficacy of cranberry extract, sodium hypochlorite and chlorhexidine as a root canal irrigant: An in-vitro study

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Abstract
INTRODUCTION. Elimination of microorganisms from the root canal system is an important consideration in endodontic treatment and hence use of irrigants with adequate antimicrobial and antifungal properties is an enormously essential factor. However, an optimal root canal irrigant remains unidentified within the current scientific literature. Herbal alternatives are garnering increasing interest due to their potential benefits, including biocompatibility, antimicrobial properties, and reduced adverse effects compared to conventional chemical irrigants.
AIM. To conduct a comparative evaluation of the antimicrobial efficacy of cranberry extract, sodium hypochlorite (5.25%), and chlorhexidine digluconate (2%) when used as root canal irrigants *in vitro* against *Enterococcus faecalis* and *Candida albicans*.
MATERIALS AND METHODS. Based on the irrigating solution used, 24 premolars were divided into 3 groups (8 in each group), Group I – cranberry extract irrigant, Group II – Sodium hypochlorite (5.25%), Group III – Chlorhexidine digluconate (2%). The teeth were sectioned at the cemento-enamel junction, and they were incubated with primary culture of E. faecalis and C. albicans and irrigated using 2ml of the respective irrigants. Pre and post irrigation microbiological sample collection were done using paper points.
RESULTS. Sodium hypochlorite as a root canal irrigant has shown highest antimicrobial efficacy against E. faecalis and C. albicans, followed by Chlorhexidine digluconate group, and the least was with Cranberry extract group.
CONCLUSIONS. Cranberry extract as a root canal irrigant has shown considerable activity against the root canal pathogens, however, is not as efficacious as sodium hypochlorite or chlorhexidine digluconate.

Keywords: root canal irrigants, Cranberry, sodium hypochlorite, chlorhexidine, plant extracts, antimicrobial agents

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Сравнительная оценка антимикробной эффективности экстракта клюквы, гипохлорита натрия и хлоргексидина в качестве ирригантов при эндодонтическом лечении: экспериментальное исследование in vitro

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Резюме
ВВЕДЕНИЕ. Устранение микроорганизмов из системы корневых каналов является важнейшей задачей эндодонтического лечения, поэтому применение ирригантов с выраженными антимикробными и противогрибковыми свойствами имеет первостепенное значение. Однако в современной научной литературе не определен оптимальный ирригант для обработки корневых каналов. На фоне этого растет интерес к растительным альтернативам благодаря их потенциальным преимуществам, таким как биосовместимость, антимикробная активность и меньшее количество побочных эффектов по сравнению с традиционными химическими средствами.
ЦЕЛЬ ИССЛЕДОВАНИЯ. Провести сравнительную оценку антимикробной эффективности экстракта клюквы, гипохлорита натрия (5,25%) и диглюконата хлоргексидина (2%) при их применении в качестве ирригантов корневого канала *in vitro* по отношению к микроорганизмам *Enterococcus faecalis* и *Candida albicans*.

МАТЕРИАЛЫ И МЕТОДЫ. В зависимости от используемого ирриганта 24 премоляра были разделены на три группы (по 8 зубов в каждой): группа I – экстракт клюквы, группа II – гипохлорит натрия (5,25%), группа III – диглюконат хлоргексидина (2%). Зубы были рассечены на уровне цементно-эмалевого соединения, инфицированы культурами *E. faecalis* и *C. albicans*, после чего обработаны соответствующими ирригантами объемом 2 мл. Микробиологические образцы до и после ирригации собирались при помощи бумажных штифтов.
РЕЗУЛЬТАТЫ. Наибольшую антимикробную активность в отношении *E. faecalis* и *C. albicans* продемонстрировал гипохлорит натрия, за ним следовал диглюконат хлоргексидина. Наименьшую эффективность показал экстракт клюквы.
ВЫВОДЫ. Экстракт клюквы проявил определенную антимикробную активность против патогенов корневого канала, однако его эффективность уступает гипохлориту натрия и диглюконату хлоргексидина.

Ключевые слова: ирриганты для корневых каналов, клюква, гипохлорит натрия, хлоргексидин, растительные экстракты, антимикробные агенты.

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INTRODUCTION

Dental caries, a prevalent chronic oral disease, often leads to pulpal and peri-apical infections requiring endodontic treatment. Endodontic infections, comprising about 40–50% of oral diseases [1], are primarily caused by microorganisms, including bacteria (65%) and fungi (30%) like Enterococcus faecalis and Candida albicans [2]. E. faecalis, found in 67% of endodontic failure cases, and C. albicans can resist traditional irrigation techniques, making effective antimicrobial treatment crucial [3]. Sodium hypochlorite (NaOCl) and Chlorhexidine are common irrigants but have limitations, such as cytotoxicity and weakening of dentin [4]. Phytotherapy, exploring natural plant extracts, has gained interest as an alternative. Cranberry (Vaccinium macrocarpon), rich in proanthocyanidins, offers antibacterial properties, inhibits Streptococcus mutans, and supports dentin-collagen cross-linkage [5].

AIM

This study aim to evaluate the antimicrobial efficacy of cranberry extract, NaOCl, and Chlorhexidine as root canal irrigants against E. faecalis and C. albicans, focusing on colony-forming unit (CFU) reduction.

MATERIALS AND METHODS

The present study was an in-vitro study, approved by the Institutional Review Board (DYPDCH/DPU/EC/412/73/2022).

Cranberry extract in crude form was obtained from Herbo Neutra Pvt. Ltd, Uttar Pradesh, India. Based on a preliminary study done to obtain the minimum inhibitory concentration of cranberry extract, 90% concentrated solution of cranberry extract was obtained by dissolving 90 grams of crude cranberry extract powder with 100 mL DMSO (dimethyl sulfoxide).

24 premolars indicated for orthodontic extractions were collected for the study. The teeth samples were freed from any tissue tags, calculus or debris by keeping them immersed in 5.25% NaOCl solution for 24 hours. Later the teeth were stored in normal saline to prevent it from dehydrating. The teeth were then sectioned from cemento-enamel junction with a carbide bur under excessive irrigation. In order to prevent bacterial leakage, the apices of the roots were glued using cyanoacrylate. They were then mounted on acrylic block for ease of instrumentation (Fig. 1).

The root canal opening was done using a small round carbide bur followed by establishing patency of the

Fig. 1. Mounted Teeth samples on acrylic blocks

Рис. 1. Зубные образцы, зафиксированные в акриловых блоках

canal using 10 K hand file (Mani). This was followed by determination of the working length using radiograph. The root canals were instrumented utilising the Step back technique and circumferential filing motions up to 35 no. K file. To remove any debris, the canals were flushed with distilled water intermittently. Each tooth was then sterilized using autoclave at 121°C for 20 minutes at 15 psi pressure. The teeth were now ready for inoculation with E. faecalis and C. albicans.

For raising the primary culture, E. faecalis (ATCC no. 29121) and C. Albicans (ATCC no. 10231) were inoculated in brain heart infusion broth after incubation at 37°C for 24 hours. The root canals of the experimental teeth were infected using a sterile insulin syringe with freshly made suspension of the organism at a concentration of 1 McFarland in order to create a standard infection in all samples. The teeth were then incubated at 37°C for 72 hrs. This is followed by collection of the baseline samples using a number 30 paper point by keeping it immersed in the root canals for 60 seconds and were then transferred in vials containing 1 mL of saline. This was then followed by microbiological culturing process.

The teeth were divided into three groups: Group I (Cranberry extract), Group II (5.25% Sodium hypochlorite), and Group III (2% Chlorhexidine digluconate), with eight teeth per group. Each canal was irrigated with 2 ml of the solution using side vented needle (NeoEndo) for 20 minutes, then flushed with distilled water. Post-irrigation, microbial samples were collected using paper points and placed in saline vials.

For microbiological culturing, the solution from the vials was homogenously streaked onto agar plates under aseptic conditions. After incubation at 37 degrees Celsius for 24 hours, plates were examined for colony growth and colonies were counted using digital colony counter. The pre and post irrigation colony count were then tabulated and compared.

Statistical analysis was conducted using SPSS v. 19. 0 (SPSS Inc, Chicago, Il,USA). The data was measured using Bonferroni post hoc test. A p value of < 0.01 was considered significant.

RESULTS

Fig. 2 depicts growth of colony forming units post irrigation in Group I, II and III.

Comparison of CFU's before irrigation showed statistically insignificant difference in all three groups ($F = 0.102$, $p = 0.9040$) and the comparison of CFU's after irrigation between all three groups using Bonferroni post hoc test showed statistically significant difference ($F = 24.364$, $p < 0.001$) (Fig. 3)

Comparison between pre and post irrigation in Cranberry extract group (Group I) showed statistically significant difference with mean difference 2.87 ($p < 0.001$). In the sodium hypochlorite group (Group II), there was statistically significant difference with a mean difference of 4.31($p < 0.001$) and in the chlorhexidine group (Group III), the mean difference was 4.50 ($p < 0.001$).

The results depict that the maximum reduction in CFU's was observed with Group II, followed by group III and last with group I.

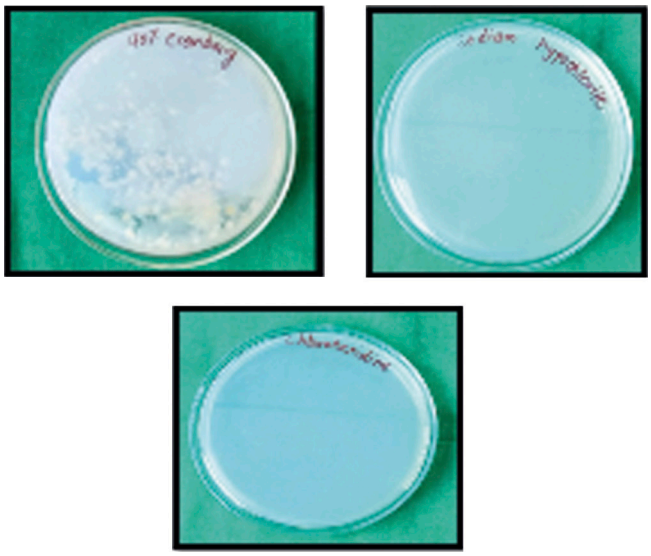

Fig. 2. Showing Colony Forming Units in Group I, Group II and Group III

Рис. 2. Колонии, образующие единицы (КОЕ) в группе I, группе II и группе III

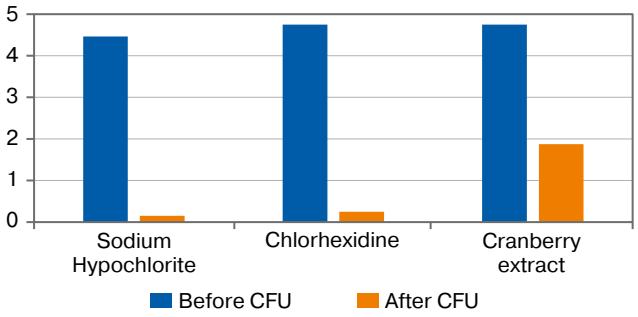

Fig. 3. Distribution and Comparison of CFU before and after irrigation with three different irrigating solutions

Рис. 3. Распределение и сравнение количества колониеобразующих единиц (КОЕ) до и после ирригации тремя различными ирригационными растворами

DISCUSSION

The primary objective of root canal treatment is to thoroughly disinfect the root canals and eliminate microbial presence. According to a study by Peter's et al., at least 35% of the root canal surfaces, including canal fins, isthmi, and cul-de-sacs, remained uninstrumented [6]. Consequently, irrigation plays a crucial role in root canal debridement as it enables cleaning beyond the extent achievable by instrumentation alone. Chemical irrigants have long been employed to effectively disinfect the root canals. Sodium hypochlorite, chlorhexidine and EDTA remain to be the most commonly used root canal irrigants [7].

Sodium hypochlorite, introduced by Henry Drysdale Dakin during World War I, is a powerful antimicrobial due to its strong oxidizing properties, effectively disrupting

microbial membranes and enzymes. However, studies, including one by Pashley, have shown that NaOCl can cause tissue necrosis, ulceration, and skin damage if used improperly [8].

Chlorhexidine, on the other hand, which is a positively charged chemical interacts with the negative charge present on the microbial cell wall and penetrates into the cell by altering the osmotic balance, thereby damaging the intracellular cell particularly adenosine triphosphate and nucleic acid [9]. Though chlorhexidine demonstrates acceptable biocompatibility, it's bitter taste can cause distortion in taste perception.

Various plant-based extracts have been used in dentistry right from tooth brushing agents, to its application as a mouthwash as well as endodontic irrigants. Bugapatti identified several commonly used herbs in dentistry, including neem, triphala, Tulsi, aloe vera, and others, in his review [10].

Cranberry, rich in proanthocyanidins (PACs), shows significant oral health benefits by inhibiting bacterial adhesion and disrupting biofilm formation. It has been explored in studies as a mouthwash, remineralizing agent, and potential root canal irrigant, offering a unique antimicrobial mechanism compared to conventional options [11; 12]. The uniqueness of cranberry PACs lies in the fact that their oligomeric molecules are of the A type, whereas most other fruits contain PACs of the B type, which lack anti-adhesion activity [13].

The root canal harbors diverse microorganisms, with E. faecalis being a major cause of initial and recurrent infections due to its ability to penetrate dentinal tubules and enter a viable but non-culturable state [14]. Though less common in primary infections, C. albicans is frequently linked to reinfections, forming biofilms that invade dentinal tissue [15].

Counting the number of Colony forming units (CFU's) using digital colony counter help us get a clear idea of the number of remnant viable cells, hence this method was employed in our study [16].

In this study, we wanted to determine whether employing a natural product like cranberry could offer comparable effectiveness to the widely used chemical irrigants such as sodium hypochlorite (NaOCl) and chlorhexidine digluconate, while mitigating the potential risks of their side effects and toxicity.

The results of this study comparatively provide valuable insights into the antimicrobial efficacy of cranberry extract compared to conventional root canal irrigants. This study revealed that all the three irrigants elicited antimicrobial properties against E. faecalis and C. albicans. Comparison of the three test irrigants depicted NaOCl and Chlorhexidine digluconate group exhibit similar range of antimicrobial activity, while Cranberry extract group exhibited comparatively lower antimicrobial properties. This is in accordance with the study performed by Tischke et al, where they found that cranberry extract irrigant was less efficacious than NaOCl and chlorhexidine digluconate, against a multispecies biofilm [17].

Cranberry extract's lower efficacy compared to sodium hypochlorite and chlorhexidine may stem from its differing mechanisms, concentration, and contact time. Optimizing cranberry formulations and protocols could improve its antimicrobial efficacy. Its natural origin may offer advantages like reduced cytotoxicity and biocompatibility, warranting further investigation into its selective pathogen targeting.

Cranberry extract's Non-Dialyzable Material (NDM) plays a key role in its antimicrobial properties by disrupting microbial biofilm attachment [13]. Additionally, cranberry components aid in dentin cross-linkage, preserving the collagen network and maintaining tooth structure strength [18]. Further SEM studies could elucidate the precise mechanism of this cross-linking effect.

While this in-vitro study highlights cranberry extract's antimicrobial efficacy, further in vivo studies with long-term follow-up are needed to determine optimal concentration and application protocols for clinical use in root canal disinfection.

CONCLUSION

In conclusion, our in vitro study highlights cranberry extract as a promising root canal irrigant with comparable efficacy to sodium hypochlorite and chlorhexidine, offering a favorable safety profile. Though sodium hypochlorite and chlorhexidine remain more effective, incorporating cranberry extract could enhance microbial control while reducing adverse effects. Further optimization of its formulations and protocols may enhance its antimicrobial activity.

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Ashrita Suvarna – a substantial contribution to the concept or design of the article.

Lalit Patil – approved the version to be published, a substantial contribution to the concept or design of the article.

Simran Deorukhkar – the acquisition, analysis or interpretation of the data for the article.

Vedashree Chaudhari – the acquisition, analysis or interpretation of the data for the article.

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Л. Патил – одобрил версию статьи к публикации, внес существенный вклад в разработку концепции и дизайна исследования.

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