Evaluation of ozonized calcium hydroxide as an effective intracanal medicament during root canal procedures: an *in vitro* observational study

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Abstract

Achieving thorough disinfection is regarded as one of the pillars in endodontics. Although calcium hydroxide (CH) is one of the routinely used intracanal medicament in endodontics; alternative approaches are gaining popularity to mitigate endodontic pathology. However, CH has to be tested for its dissociation which is a rate-limiting attribute essential for its therapeutic action. The dissociation of CH into OH^- and Ca^{2+} depends on the vehicle used to prepare the paste. This *in-vitro* study evaluated the use of ozonized olive oil in facilitating calcium ion release and change in pH when combined with CH. Fifty single rooted extracted human mandibular premolars were instrumented with NiTi rotary files (40/6). The teeth were divided into two groups ($n = 25$ per group) on the basis of vehicle: olive oil (CH + olive oil) and ozonized olive oil (CH + ozonized olive oil) groups. Both olive and ozonized olive oil vehicles allowed the diffusion of ions. However, pastes prepared with ozonized oil showed more ion diffusion, with marked calcium ion release after 15 days and alkalinity was maintained for complete period of 15 days, depicting better support for CH action. The change in calcium ion release and alkalinity were statistically significant in ozonized oil vehicle compared to olive oil vehicle. The present *in-vitro* study supports the use of ozonized olive oil as a vehicle to be used with CH as an intracanal medicament, considering its anti-microbial potential and sustainable release of calcium ions. The study was approved by the Institutional Ethical Committee of Manubhai Patel Dental College (approval No. MPDC_130/CONS-25/17) on June 4, 2018.

Key words: atomic absorption spectrophotometer; calcium hydroxide; Candida albicans; endodontic microflora; endodontics; Enterococcus feacalis; intracanal disinfection; olive oil; ozonized olive oil; root canal therapy

doi: 10.4103/2045-9912.296042
How to cite this article: Vasavada K, Kapoor S. Evaluation of ozonized calcium hydroxide as an effective intracanal medicament during root canal procedures: an *in vitro* observational study. Med Gas Res. 2020;10(3):122-124.

Introduction

Endodontics is one of prime branch of dentistry that deals with the issues related to dental pulp and periapical tissues. Root canal treatment (RCT) is commonly performed by the endodontists for mitigating infected root canals. It is a known fact that there are diverse floras of bacteria present in the infected roots which are penetrated in to the dentinal tubules where the host defense mechanisms are compromised.[1,2] RCT is mainly aimed at cleaning the root canal system and protecting the root canal against any possible re-infection which will accelerate the healing process.[2] Intracanal medicaments are utilized in endodontics to achieve this feat of complete seal. Besides offering disinfection, these medicaments also help in achieving rapid pain relief post the treatment.[3] Calcium hydroxide (CH) remains as the first choice intracanal medicament owing to its bactericidal property, stability and capacity to stop inflammatory exudates compared to other medicaments especially in multiple-visit endodontics.[4]

These favorable actions of CH are attributed to its alkaline pH (12.5–12.8) and presence of calcium ions. The former denatures and detoxify the bacterial enzymes, whereas the later is important for tissue mineralization by stimulating the fibronectin gene expression.[5,6] Further, the dissociation of CH in to calcium and hydroxyl ions is another rate-limiting step in making CH as a dentist's choice of intracanal medicament.[7] In order to achieve effective dissociation of ions, appropriate choice of a vehicle is warranted. Earlier studies have shown that the velocity of dissociation of these ions at the periapical tissues and root canal is greatly influenced by the vehicle used to prepare the paste.[8,9] Although there are many types of vehicles available, oily vehicle-containing paste is employed in clinical situations like chronic long standing lesions and inflammatory root resorption that necessitates gradual, uniform and slow ionic liberation. Olive oil is one among such mediums which is used to prepare CH paste owing to its availability and affordability.[9,10]

In order to achieve optimum results through an RCT, the anti-microbial capacity of the pastes used is also taken in to consideration. Utilization of medical ozone in various forms like ozonated water, ozonated oil or gas is gaining popularity among the dentists. This is mainly due to ozone's efficacy in achieving faster healing and its anti-microbial properties.[11] Use of ozonated oil has shown its efficacy as an antimicrobial agent especially on the bacterial species associated with periradicular diseases.[12] As discussed earlier, besides anti-microbial property, determining the dissociation potential of the vehicle is also an essential step that determines the therapeutic efficacy of any vehicle.[13,14] However, there are no such studies available to understand whether ozonated oil based pastes can offer better ionic distribution compared to non-ozonized oil pastes.

The present study is an *in-vitro* analysis to understand the ionic dissociation and pH level of CH when combined with indigenous vehicles (ozonated *vs*. non-ozonated olive oil).

Materials and Methods

The study was conducted at the Department of Conservative Dentistry and Endodontics and was approved by the Institutional Ethical Committee of Manubhai Patel Dental College (approval No. MPDC_130/CONS-25/17) on June 4, 2018. Fifty single-rooted premolar teeth extracted from the consented patients for orthodontic purpose/periodontal disease were used for this study. The extracted teeth did not have any gross caries/fractures and having patent canals and intact full length root were selected for the study. Any teeth with calcification within the canal, resorptions and slightest deviation from the normal anatomy were excluded from the selection.

Specimen preparation

The extracted teeth were disinfected using 10% formaldehyde which were later debrided by using an ultrasonic scaler, the crowns of all the teeth were decoronated using a diamond disc; post radiographic confirmation for the patency of the canal. The length of each specimen was standardized to 15 mm and orifice openers were used to enlarge the root canal orifices. A size 10 K file was placed in the canal to an extent it was visible at the apex. The working length was then determined to be 1 mm short of this length.

Root canals were enlarged up to size 40 apically with 6% taper using NiTi rotary files (Orikam Healthcare, Gurugram, India). Irrigation was accomplished by 2 mL of 2.5% Sodium hypochlorite (NaOCl) solution (Prime Dental Products, Bhiwandi, India) and 2 mL of 17% ethylene-diamine-tetraacetic acid (Prime Dental Products). Further 5 mL of distilled water was used for final irrigation. Each specimen was stored in 5 mL of saline before the experiment.

The teeth were randomly divided into two groups with 25 teeth in each group. Group one comprised of those teeth with 1 mg of CH (Vishal Dentocare Pvt. Ltd., Ahmedabad, India) mixed in 2 mL of olive oil (Figaro Olive Oil™, Deoleo, S.A, Spain) whereas group two was those teeth with 1 mg of CH (Vishal Dentocare Pvt. Ltd.) mixed 2 mL of ozonated oil (Ozonized Olive Oil™, Ozone Forum India, Bisleri, Mumbai, India). Lentulospirals (Mani™paste carriers) were used to place the formulations in to the root canal.

The root canal orifices were sealed with sticky wax and the teeth were painted with nail paint except in the apical third region to seal lateral canals present in coronal and middle third region of the root. The prepared samples were then immersed into 50 mL distilled water and were kept in an incubator at 37°C, with 100% humidity.

Outcome measures

The change in pH of the distilled water was determined using Digital pH meter (ANALAB scientific instruments-model pH Cal10, Gujarat, India) and for calcium ion analysis 10 mL of sample for analysis was then withdrawn periodically at 24 hours, 72 hours, and 7 days and 15 days. The solutions were analyzed for calcium ion release with Atomic absorption spectrophotometer (SHIMADZU AA-6680, Kyoto, Japan).

Statistical analyses

All the analyses were carried out through SPSS version 20.0 statistical Analysis Software (IBM, Armonk, NY, USA). Both descriptive and inferential analysis deployed to understand the mean difference within as well as between the two groups. Independent samples *t*-test were used to find the difference between the ozonized *vs*. olive oil vehicles.

Results

The results compared the mean difference in the calcium ion release and changes in pH using descriptive statistics. From 24 hours to 15 days, there was gradual increase in calcium ion release recorded in both the groups. However, ozonated olive oil group exhibited maximum release of calcium ions in all the assessment intervals except day 1. There was a statistically significant change in the ozonized olive oil group compared to olive oil group for calcium ion dissociation across 24 hours, 72 hours, 7 days and 15 days respectively ($P < 0.001$; **Table 1**).

The changes in pH were predominant in olive oil group immediately after 24 hours with mean pH reported to be 9.54. This further rose significantly on the 15th day as 10.00, which signifies that the alkalinity was maintained throughout the experimental period. In ozonized olive oil group, maximum pH dissociation was obtained only after 72 hours with mean pH of 9.58. At the end of 15th day dissociation of pH in ozonized olive oil group was 10.00. There was a statistically significant change in the ozonized olive oil group compared to olive oil group for pH across 24 hours, 72 hours, 7 days and 15 days respectively ($P < 0.001$). The detailed results are tabulated in **Table 2**.

Table 1: Calcium ions release (ppm) in teeth treated by olive oil and ozonized olive oil

Group	24 h	72 h	7 d	15 d
Olive oil	6.80±0.94	9.84±1.00	10.48±0.91	9.55±0.69
Ozonized olive oil	5.33±0.38***	12.67±1.53***	19.58±1.94***	20.62±1.77***

Note: Data are expressed as the mean ± SD, and were analyzed by independent samples *t*-test. ***$P < 0.001$, *vs*. olive oil group. ppm: Parts per million.

Table 2: pH level in teeth treated by olive oil and ozonized olive oil

Group	24 h	72 h	7 d	15 d
Olive oil	9.54±0.29	9.58±0.23	9.80±0.22	10.00±0.31
Ozonized olive oil	7.51±0.23***	8.22±0.33***	8.80±0.40***	10.00±0.52***

Note: Data are expressed as the mean ± SD, and were analyzed by independent samples *t*-test. ***$P < 0.001$, *vs*. olive oil group.

Discussion

CH has wide utility in endodontics owing to its anti-microbial and biological activity. Diffusion of calcium ions and higher pH levels are regarded as the rate limiting factor determining the anti-microbial effect of CH.[15] Studies warrant utilization of appropriate vehicle to prepare the CH paste and placing it in the root canal has greater therapeutic value in RCT.[16] This study demonstrates that treating olive oil (vehicle) with ozone enhances the anti-microbial property and alkalinity of CH.

Though routine CH paste has a wide range of clinical applications, but it is less effective against specific species such as Enterococcus faecalis and Candida albicans responsible for root canal failures.[17] This necessitates the use antimicrobial vehicles with CH because of its relative inefficiency against some microorganisms.[18,19] Ozone-due to its anti-oxidant properties and anti-microbial properties is widely recommended as an adjunct to primary root canal disinfectants.[20]

Viscous vehicles have higher clinical value as they not only offer greater healing but also reduce the number of dental visits especially in cases of chronic inflammation and large periapical lesion.[15] When olive oil is ozonated suitably and sufficiently, density of oil increases in direct proportion with ozonation time.[21] So, together with an effective ozonation process, it will be more viscous than extra virgin olive oil, with increased viscosity there is slower ionic liberation which supports our result that dissociation started only after 72 hours in case of ozonized oil contrary to olive oil in which dissociation was recorded at 24 hours. Furthermore, the alkalinity was maintained for 15 days and there was continual, slow and gradual release of calcium ions even after 15 days.

Further the release of calcium ions was higher in the ozonated olive oil group which is suggestive of higher mineralization activity post-ozonation. The anti-microbial activity of ozonated olive oil can be inferred from the sustained alkaline state of the distilled water. Though both the groups in this *in-vitro* study have shown gradual increase in calcium ion and pH from first day to 15 days, the higher dissociation was observed on the 15th day. This is suggestive of longer duration required for oil based dressings to achieve optimum results. Further, the dissociation was more predominant in ozonated olive oil dressing. These findings warrant utilization of ozonated olive oil with CH as an intracanal medicament. Further *in-vivo* studies are needed to authorize ozonated olive oil-CH paste as a potential tool in mitigating infections and promote healing in RCT.

Ozonated olive oil-CH paste as demonstrated in this *in-vitro* study exhibits slower and gradual release of ions compared to olive oil based CH dressing in infected root canals. It is suggestive that ozonized oil based CH dressing can be used in cases of chronic large periapical lesions with or without bone destruction, where long term dressing of CH is required to induce mineralization activity and provide long term antimicrobial environment.

Author contributions
All the authors has substantially contributed in the design and conduct of the study, manuscript preparation, analysis, and reporting of this study.
Conflicts of interest
None declared.
Financial support
None.
Institutional review board statement
The study was approved by the Institutional Ethical Committee of Manubhai Patel Dental College (approval No. MPDC_130/CONS-25/17) on June 4, 2018.
Declaration of patient consent
The use of teeth was under the patients' informed consent.

Data sharing statement
Datasets analyzed during the current study are available from the corresponding author on reasonable request.
Plagiarism check
Checked twice by iThenticate.
Peer review
Externally peer reviewed.

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Received: April 22, 2020
Accepted: June 5, 2020
Published Online:September 30, 2020