Comparative Evaluation of Chlorhexidine and Cinnamon Extract used in Dental Unit Waterlines to Reduce Bacterial Load in Aerosols during Ultrasonic Scaling

Abstract

Background: Dental unit waterlines (DUWL) are believed to be a source of infection. Ultrasonic instruments generate aerosols with significantly greater numbers of bacteria. Chlorhexidine (CHX) exhibits significant antiseptic effect. Recently, cinnamon (CIN) has been displayed to have antibacterial and anti-inflammatory properties in vivo. **Aim:** The aim of this study is to compare and evaluate the efficacy of CHX versus CIN extract in the reduction of bacterial count in dental aerosols when used as an irrigant through DUWL during ultrasonic scaling. **Materials and Methods:** Sixty patients with moderate-to-severe gingivitis were randomly divided into 3 groups of 20 patients each undergoing ultrasonic scaling. For experimental group I, CHX was used in dental unit reservoir before ultrasonic scaling. Similarly, in group II, CIN extract was used and group III served as control where distilled water (DW) was used. The aerosols from ultrasonic units were collected on two blood agar plates at three different positions. One plate from each position was incubated aerobically for 48 h and other plate anaerobically for 72 h. The total number of colony forming units (CFUs) was then calculated and statistically interpreted. **Results:** CHX and CIN both were equally effective ($P > 0.05$) in reducing the bacterial count in aerosols as compared to DW ($P < 0.05$) when used through DUWL. Maximum contamination was seen on the agar plate placed at the chest of the patient. **Conclusion:** Both CIN and CHX used as an irrigant through DUWL effectively helped in the reduction of bacterial count in dental aerosols.

Keywords: Aerosols, agar plate, chlorhexidine, cinnamon, dental unit waterlines

Introduction

Dental personnel are taking steps toward controlling the transmission of diseases during various dental procedures. During dental treatment, saliva may become aerosolized and microorganisms from the oral cavity may contribute to the spread of infection.[1] It has been confirmed from the past literature that the use of ultrasonic and sonic scalers, air polishers, and air turbines produce aerosols. As aerosol-creating instruments have been shown to be the main cause of this potential cross-contamination, there is a constant endeavor to quantify this environmental hazard. The propelling force of a high-speed dental drill and the cavitation effect of an ultrasonic scaler, both being used in combination with a water spray, can generate numerous airborne particles derived from blood, saliva, tooth debris, dental plaque, calculus, and restorative materials.[2]

Aerosols are defined as suspensions of liquid and/or solid particles in the air generated by coughing, sneezing, or any other act that expels oral fluids into the air.[3] Although there are several different definitions in the literature, aerosols containing particles more than 50 mm in diameter are referred to as spatter, whereas particles measuring less than 50 mm are called droplet nuclei. Because gravitational pull causes spatter aerosols to settle very quickly on surfaces, they are less likely to carry microorganisms that induce infection. Droplet nuclei, however, remain suspended in the air for many hours and can infect persons by direct inhalation and penetration deep into the lungs. Larger 10–15-mm droplet nuclei particles are closely related to upper respiratory infections, whereas smaller 0.5–5 mm droplet nuclei can accumulate in the lower respiratory tract and may cause viral respiratory infections.[4,6]

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Dental unit waterlines (DUWL) have demonstrated to be the sites for the development of biofilms of aerobic, mesophilic, and heterotrophic microorganisms which are commonly found in fresh drinking water systems. Dental units contain many fine-diameter tubings. The inside diameter of these tubings is about 1–2 mm; so the intraluminal surface-to-volume ratio is greater than that in the water mains and pipes that bring water to the units. The flow of water in the mains, pipes, and tubings is laminar; therefore, the flow at the lumen surface is almost at a standstill. In this zone, the bacteria may move by fluid flow, Brownian motion, sedimentation, and flagella. Molecules in the water may adhere to the lumen surface by physical adsorption and chemisorption, providing a conditioned substratum that can attract other molecules projecting from the surface of the microorganisms by means of van der Waal’s forces, electrostatic forces, hydrophobic forces, or chemisorption of bacterial fimbriae, pili, or adhesins.

Most DUWL biofilms (30–50 μm thick) are enveloped in a polysaccharide slime layer known as a glyocalyx, which provides resistance to chemical agents. As they colonize and grow, clumps of microbes break off and become free floating (“planktonic”) in the waterline. The next time the water is discharged from the unit, the free-floating colonies leave the waterline and end up in the patient’s mouth. Studies show that contaminated dental water poses a risk to dental professionals, because the dental procedures generate large amounts of aerosols that might be inhaled. Some evidences suggest direct link of cross-infection to the contamination in DUWL. Hence in the present study, chlorhexidine (CHX) and cinnamon (CIN) were used as antimicrobial agents through DUWL.

Various authors have given different treatment modalities for the treatment of DUWLS, but no definite solution has been introduced yet. Meiller et al. showed that sodium hypochlorite, cavitide, glutaraldehyde, listerine antiseptic, peridex, and sterilex ultra are potentially useful in the management of DUWL biofilm. Studies show that 0.2% CHX has a significant antiseptic effect and that CIN has antibacterial, anti-inflammatory, and antifungal properties. The proposed mechanism of antimicrobial action of CIN may be due to the presence of cinnamaldehyde, an aromatic aldehyde, which is highly electronegative and interferes in biological processes involving electron transfer and reacts with nitrogen containing components, for example, proteins and nucleic acids, and therefore inhibits the growth of the microorganisms.

As aerosols are potentially hazardous and are involved in the spread of infection, their reduction should be contemplated. To the best of our knowledge, this is the first study for aerosol reduction which uses CIN as an antimicrobial agent through DUWL as an ultrasonic irrigant and to compare it with CHX. Therefore, the aim of this study was to evaluate, aerobically and anaerobically, the efficacy of a 0.2% CHX mouthwash, and a freshly prepared solution of CIN extract, used as an ultrasonic irrigant through DUWLS, in reducing microorganisms in dental aerosols.

Materials and Methods

This single-center, three-group parallel-designed study was conducted over a period of 3 months. The subjects enrolled in this study were selected from the Outpatient Department of Periodontology. Ethical clearance was obtained from institutional ethical committee and guidelines of declaration of Helsinki were strictly followed. A written informed consent was signed by all the patients.

Sample-size calculation

The sample size was calculated to check for changes in the CFUs. This was done by fixing α error at <5% (P < 0.005). Based on this calculation, the minimum sample size required in each group was 20 subjects. Subjects were enrolled in three groups.

Patient selection

The patients included in the study were initially screened for their gingival index (GI) and plaque index (PI) scores in the first sitting [Table 1]. A total of 60 subjects having moderate-to-severe gingivitis from both the sexes with age ranging from 18 to 55 years (mean ± SD of 29.6 ± 7.6), willing to participate in the study, and having a GI score of 2–3 and a PI score of 2–3, were selected for this study. The patients were randomly allotted to one of the three groups by one examiner (KS) while the treatment was performed by another examiner (AM) [Figure 1].

Inclusion criteria

A. Subjects having minimum of 20 permanent teeth
B. Moderate-to-severe gingivitis, that is, a GI score of 2–3
C. Systemically healthy patients
D. Subjects indicated for full-mouth scaling in single sitting.

Exclusion criteria

A. The presence of any systemic disease
B. Received antibiotics or nonsteroidal anti-inflammatory drugs in the past 3 months
C. Oral prophylaxis within last 3 months
D. Pregnant and lactating mothers
E. Smokers.

Table 1: Mean values of gingival index and plaque index expressed as mean±standard deviation

|                | CHX group          | CIN group         | DW group          |
|----------------|--------------------|-------------------|-------------------|
| Gingival index | 2.51±0.223        | 2.5±0.2261        | 2.46±0.3688      |
| Plaque index   | 2.56±0.2011       | 2.4±0.2108        | 2.63±0.3057      |

CHX=Chlorhexidine, CIN=cinnamon, DW=distilled water
Preparation of cinnamon extract

Cinnamon extract was prepared using the formulation used earlier by Gupta D et al (2015). Fresh CIN bark was taken from the Botanical Garden. It was ground to a fine powder in a mechanical grinder. Ten grams of finely powdered CIN were mixed with 100 ml of sterile deionized water and kept in a water bath in a round-bottomed flask at 55–60°C for 5 h, then filtered through sterile filter paper (Whatman®, United Kingdom). The aqueous extract was decanted, clarified by filtration through a muslin cloth, and evaporated in a porcelain dish at 40°C, which resulted in the dried extract. This dried extract was suspended in polyethylene glycol 400 (20% w:v) and sterile distilled water (DW) to give a final concentration of 20% w:v. The entire procedure was performed under proper aseptic conditions.

Clinical procedure

All the ultrasonic procedures were carried out in a closed operatory with the facility to fumigate the room. Prior to the procedure, the surfaces of the operatory were disinfected with ethyl alcohol (70%). Before starting the procedure, the ultrasonic unit was switched on and flushed for 2 min in order to get rid of contaminated water due to overnight stagnation in waterlines. Thirty minutes prior to the procedure, a blood agar plate was positioned on the plate 1 spot for a period of 20 min. This was then subjected to microbial assessment in order to check for environmental contamination, if present, in the operatory. The procedure commenced only after the operator was assured that there is no environmental contamination seen on the agar plate.

Sixty patients who met the inclusion criteria were selected. The type of procedure to be performed and the likely discomfort was fully explained, and written informed consent was obtained from each patient. Patients were randomly allocated to one of the following three groups: CHX (Group I), CIN (Group II), and DW (Group III).

Dental chairs with self-contained water system were selected for the study. The abovementioned agents were added in the DUWL. Strict asepsis was observed inside the operatory, and the selected subjects were prepared to enter the operatory by wearing headcaps and autoclaved gowns. The subjects were instructed to abstain from all the actions that generate aerosols. Various actions such as conversation, sneezing, and coughing were strictly forbidden (if any such action occurred incidentally, then that subject was excluded from the study). Single sitting ultrasonic scaling was done for all the patients for a period of 20 min, by using Woodpecker UDS-P Piezo Ultrasonic scaler. During each scaling procedure, saliva ejector was used.

Position of agar plates

Blood agar was chosen because it is a general purpose, nonselective and enriched medium, which promotes the growth of microorganisms, such as those sampled from air. Table 1 shows the three standardized locations of the blood agar plates placed in operatory room for each treatment group and fixed distances of the plates were also maintained with respect to the reference point, that is, the mouth of the patient [Table 2 and Figure 2].

Microbial analysis

The aerosols from the ultrasonic unit were collected on two blood agar plates placed at three different positions, each within a range of 1 ft, in all the three groups. After collecting the samples, one plate from each position was incubated aerobically for 48 h and other plate anaerobically for 72 h. Anaerobic culture was carried out using BD

![Figure 1: Study flow chart](image1)
![Figure 2: Schematic representation of the position of agar plates](image2)

**Table 2: Distances of agar plates from patient’s mouth**

| Plate number | Plate position                              |
|--------------|--------------------------------------------|
| Plate 1a, 1b (C) | 1 ft from patient’s mouth at patient’s chest |
| Plate 2a, 2b (R) | On the right side of patient’s mouth at a distance of 1 ft |
| Plate 3a, 3b (L) | On the left side of patient’s mouth at a distance of 1 ft |

C=Chest, R=right, L=left
GasPak™ EZ Pouch Systems in which agar plates were kept and incubated for 72 h. Colonies of bacteria were counted using classical bacterial counting technique and they were expressed as a number of CFUs (colony forming units) seen on agar plates.

**Statistical analysis**

Statistical analysis of the results was done for CFUs, PI, and GI using SPSS software version 20 (Chicago, IL, USA). The ANOVA test was used for continuous variables after confirming normality of the data distribution. The method of Bartlett was used to confirm that the data had a Gaussian distribution. Statistical significance was defined as $P < 0.05$.

**Results**

**Microbial analysis**

**Aerobic analysis**

CFUs seen in the CHX group (Group I) at chest, right and left, were 608.0 ± 74.803, 450.0 ± 66.625, and 440.5 ± 60.803 (mean ± SD), respectively. CFUs seen in the CIN group (Group II) at chest, right and left, were 575.5 ± 80.638, 419.5 ± 48.215, and 413.5 ± 51.373 (mean ± SD), respectively. CFUs seen in the DW group (Group III) at chest, right and left, were 1422.0 ± 109.42, 1074.0 ± 73.967, and 1008.0 ± 58.080 (mean ± SD), respectively [Table 3 and Figure 3].

**Anaerobic analysis**

CFUs seen in the CHX group (Group I) at chest, right and left, were 361.80 ± 15.203, 244.10 ± 18.621, and 245.30 ± 17.243 (mean ± SD), respectively. CFUs seen in the CIN group (Group II) at chest, right and left, were 418.90 ± 22.776, 279.80 ± 27.80, and 266.10 ± 15.087 (mean ± SD), respectively. CFUs seen in the DW group (Group III) at chest, right and left, were 680.0 ± 42.492, 379.50 ± 25.357, and 365.50 ± 26.406 (mean ± SD), respectively [Table 4 and Figure 4].

Both aerobically and anaerobically, statistically significant difference was seen in the CFUs at chest versus right and chest versus left agar plate positions in all the three groups while the difference was not statistically significant for right versus left agar plate positions in all the three groups. Maximum aerosol contamination was seen on the agar plate placed at the chest of the patient.

**Discussion**

Aerosols produced during various dental procedures have the potential to spread infection to dental personnel and other individuals in dental clinic. Two major sources of aerosol contamination are oral microbial flora in patients because of the suction and backflow of patient’s saliva from the saliva ejector and possibility of entering the water system and biofilm in waterlines.[7] During treatment, aerosols can spread rapidly in the indoor air and can affect the microbiological quality of the air. According

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**Table 3: Aerobic analysis: Colony forming units counted on blood agar plates (values expressed as mean±standard deviation)**

|          | CHX group | CIN group | DW group | Significance                  |
|----------|-----------|-----------|----------|-------------------------------|
| Chest    | 608.0±74.803 | 575.5±80.638 | 1422.0±109.42 | CHX versus CIN - $P>0.05$               |
|          |           |           |          | CHX versus DW - $P<0.001$*       |
|          |           |           |          | CIN versus DW - $P<0.001$*       |
| Right    | 450.0±66.625 | 419.5±48.215 | 31,074.0±73.967 | CHX versus CIN - $P>0.05$               |
|          |           |           |          | CHX versus DW - $P<0.001$*       |
|          |           |           |          | CIN versus DW - $P<0.001$*       |
| Left     | 440.5±60.803 | 413.5±51.373 | 1008.0±58.080 | CHX versus CIN - $P>0.05$               |
|          |           |           |          | CHX versus DW - $P<0.001$*       |
|          |           |           |          | CIN versus DW - $P<0.001$*       |

*Means significant. CHX=Chlorhexidine, CIN=Cinnamon, DW=Distilled water
Table 4: Anaerobic analysis: Colony forming units counted on blood agar plates (values expressed as mean±standard deviation)

|                | CHX group  | CIN group  | DW group  | Significance                  |
|----------------|------------|------------|-----------|-------------------------------|
| Chest          | 361.80±15.203 | 418.90±22.776 | 680.0±42.492 | CHX versus CIN - P<0.05       |
|                | CHX versus DW - P<0.001* | CIN versus DW - P<0.001* | CIN versus CIN - P<0.001* |
| Right          | 244.10±18.621 | 279.80±27.80    | 379.50±25.357 | CHX versus CIN - P<0.05       |
|                | CHX versus DW - P<0.001* | CIN versus DW - P<0.001* | CIN versus CIN - P<0.001* |
| Left           | 245.30±17.243 | 266.10±15.087 | 365.50±26.406 | CHX versus DW - P<0.001*       |

*Means significant. CHX=Chlorhexidine, CIN=Cinnamon, DW=Distilled water

to the recommendation of American Dental Association, potentially contaminated aerosols or splatter should be controlled during various dental procedures.[19]

DUWLs harbor appreciable amounts of bacteria that are derived from the biofilm on the inner surface of these lines. This continuous reservoir of bacteria carries the potential of causing infection to patients and dental workers. Opportunistic pathogens such as *Pseudomonas, Legionella, Candida, Penicillium,* and *Aspergillus* can be found in DUWLs. These pathogens can lead to serious systemic conditions, such as common cold, influenza, tuberculosis, HBV, HIV, and legionellosis.[20]

As the pathogens show a high probability of bypassing the host defense, a need of an adjunctive therapy in the form of chemical plaque is often warranted, in order to reduce the bacterial load in the aerosol.[21] It has been suggested that chemical treatment protocol could be used intermittently as a “shock” treatment or the chemical can be continuously introduced into waterlines in small quantities. This protocol requires having an independent reservoir system from which the solution of choice can be originated.[22] Earlier studies have shown that CHX can be used in the management of DUWL biofilm.[23] Taking this into consideration, chemicals, that is, CHX 0.2% and CIN extract 20% w: v were used in the present study through DUWLs.

CIN (*Cinnamomum zeylanicum*) is a member of the Lauraceae family. It is one of the main herbs to be used extensively for treatment of several conditions. CIN is used in dried form or ground form. The bark of the CIN tree contains an essential oil called cinnamaldehyde, which gives CIN its characteristic flavor and aroma. The inner bark of the CIN tree has been used as a spice for thousands of years. CIN is thought to have many health benefits, so it is used as an herbal medicine. From historical time, CIN has been used as a medicine for colds, flatulence, nausea and diarrhea by improving energy, vitality, and circulation.[24,25]

CIN is effective in inhibiting the growth of both gram-positive and gram-negative bacteria.[24] Its antimicrobial activity is attributed to *ω*-methoxycinnamaldehyde. Gupta and Jain[18] evaluated the antimicrobial and anti-inflammatory properties of CIN-containing mouthwash and concluded that CIN has similar effects on plaque and gingivitis compared to the bench mark control, CHX. Similar results were obtained by previous investigators.[24] Hence, in the present study, CIN extract was used as one of the antimicrobial agents through DUWLs.

Milejczak[26] determined how far the aerosols travel by performing sonic and ultrasonic scaling for 20 min. The results of the study demonstrated that particulate concentrations were present for 240 cm (nearly 8 ft). The greatest concentration of particles was present at the end of the procedure and a mean aerosol amount of 0.022 units was still present 2 h after the procedure. The greatest amounts of aerosols were found during all time trials in the 30–90-cm range (1–3 ft), which is in the operators’ work zone. Hence in the present study, the agar plates were kept at a distance of 1 ft from the patient’s mouth.

To the best of authors’ knowledge, no study has demonstrated the use of CHX and CIN extract through DUWLs in the reduction of bacterial load in dental aerosols. The primary motive of using these agents through DUWL is that these agents will disinfect the biofilm formed in the DUWL, and by doing so, the aerosols collected on the agar plate will be only from the patients mouth.

Additional studies with larger sample size are needed to validate these findings. Longer duration of evaluation will give more predictable results and would confirm its stability. Moreover, the “fall out” or plate count approach which is used to recover viable bacteria is subjected to a level of error because bacteria exposed to the air may remain viable; yet, they may lose the ability to form colonies, that is, they become non-culturable. If some airborne bacteria exhibit this phenomenon, data from colony formation will underestimate the true extent of bacterial populations in air samples.
Conclusion

Within the limitations of this study, both CIN and CHX used as an irrigant through DUWLs effectively helped in the reduction of bacterial contamination in dental aerosols which were seen by reduction in the CFUs, after adding these agents in the DUWL. CIN extract can be used as an antimicrobial agent in various disciplines of dentistry with future studies evaluating its action in different situations. Moreover, its low cost may motivate the patient at especially low socioeconomic strata for oral hygiene maintenance. This is an encouraging result, which clearly favors the promotion of CIN among the rural communities, especially belonging to low socioeconomic strata, as CIN is easily available, inexpensive, and a safe alternative to CHX. In addition, as the best line of action is prevention of the disease-causing entity, and thereby disease itself, these agents can be promoted to be used through DUWLs.

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Conflicts of interest

There are no conflicts of interest.

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