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Comparative Evaluation of the Cardamom and Chlorhexidine Extract as an Ultrasonic Coolant for Reduction of Bacterial Load in Dental Aerosols

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Abstract

Introduction: The term aerosol as defined by Micik et al includes particle size of 50µm or less, while splatter includes particle greater than 50µm.The use of various dental equipment such as dental handpieces, ultrasonic or sonic scalers generate aerosols and splatter containing microorganisms from the external environment. These aerosols or splatter contain microorganisms which may potentially cause cross contamination and infect the dental office and health. Chlorhexidine has emerged as a gold standard, its use leads to certain side effects such as dryness and soreness of mouth and alteration in taste. Cineole a major component of cardamom oil is an antiseptic that can kill bad breath bacteria. A study concluded that the cardamom extracts showed antimicrobial activity against oral pathogens like streptococci mutans and Candida albicans. Its slightly pungent but pleasant taste stimulates salivary flow. This application has not yet been investigated for cardamom extract which is known to have antibacterial and anti inflammatory properties in vivo.

Material and Methods: 30 patients diagnosed with chronic gingivitis in respective college were randomly divided into 3 groups of 10 patients each undergoing ultrasonic scaling. For Group I, chlorhexidine was used as an ultrasonic coolant; for Group II, cardamom extract was used; and Group III was served as control where distilled water (DW) was used. The aerosols from ultrasonic units were collected on two blood agar plates at different positions at baseline and 3months follow-up.

Conclusion: Both cardamom and chlorhexidine when used as an ultrasonic coolant effectively helped in the reduction of bacterial contamination in dental aerosols

which was seen by reduction in the colony forming unite counts.

Keywords: Periodontal, Dental Handpieces, Elettaria Cardamomum

Introduction

Periodontal disease is multifactorial in nature and the oral cavity harbors millions of bacteria and viruses from the respiratory tract, saliva, and dental plaque. These microorganisms get aerosolized when they come in contact with the dental equipment.

Spread of infection through splatter and aerosol has been considered a major risk factor for the dental professionals because of transmission of the infection from the patient to health care providers.Various dental equipment such as the dental handpieces, air–water syringes, ultrasonic scalers, and air polishing units are known to produce the aerosols during dental procedures, and the published data indicate that they produce many folds increase in colony forming units (CFUs) when compared pre- and post-operatively.(1)

Aerosols are the suspension of liquid or solid particles containing viruses and bacteria which are suspended in gas for few seconds.[(1)] The size of the particle may vary from 0.001 mm to more than 100 μm. The smallest particle size (ranging between 0.5 μm and 10 μm) has the greatest potential to penetrate the respiratory passages and the lungs, possessing the ability to transmit the disease.[(2)]

Miller concluded that aerosols generated from the patients mouth contain millions of bacteria per cubic foot of air. King et al. reported that bacteria could be recovered within 6 inches from the mouth of patient and the CFUs formed were significantly reduced when aerosol reduction device was used. However, the use of water as an ultrasonic coolant during debridement procedures increases the chances of aerosol productions, the risk of cross-contamination, and also the chances of transient bacteremia. Thus, to avoid this, various chemotherapeutic agents have been utilized as a coolant. The use of antimicrobials as a coolant serves the dual purpose of not only reducing the bacterial count in aerosols but also helps in continuous irrigation of the treatment site which further enhances the gingival health.

Cardamom is the dried, unripened fruit of the perennial plant species “Elettaria cardamomum”. It is also invaluable for its medicinal properties. The principal constituents of the volatile oil are cineol, terpinene, limonene, sabinene, and terpineol in the form of formic and acetic acid. Cineole a major component of cardimum oil is an antiseptic that can kill bad breath bacteria and various others. This study is the first to provide evidence that cardamom extracts through their antibacterial and anti-inflammatory properties may be therapeutic agents of interest against periodontal infections.[(3)]

Although various antimicrobials have been tried and tested conventionally as mouthrinses, chlorhexidine has emerged as a gold standard. However, its use leads to certain side effects such as staining of teeth and restorations, dryness and soreness of mouth, increased supragingival calculus formation, and alteration in taste. Apart from chlorhexidine, other agents which have been used as ultrasonic device cooling agent include povidone-iodine.[(4)]

Materials and Methods

This was a single-center, randomized clinical trial with a three-group parallel design. The study was conducted over a period of 3 months, and participants enrolled were selected from the outpatient department of periodontology. Ethical clearance was obtained from the Institutional Ethical Committee. A written informed consent was signed by all the patients.

Sample size calculation

The sample size was calculated for α error fixed at <5% ($P < 0.005$). Based on this calculation, the minimum sample size required in each group was 22 participants. Participants were enrolled in three groups.

Selection criteria

The inclusion criteria of this study were as follows:

(i) Systemically healthy patients,

(ii) Participants diagnosed with established chronic gingivitis

(iii) Participants having minimum of 20 permanent teeth,and

(iv) Participants indicated for full-mouth scaling in single sitting.

The exclusion criteria of this study were as follows:

(i) The presence of any systemic disease,

(ii) Received antibiotics or nonsteroidal anti-inflammatory drugs in the past 9–11 weeks,

(iii) Oral prophylaxis within the past 3 months,

(iv) Pregnant and lactating mothers, and

(v) Smokers.

Patient selection and randomization-

A total of 66 participants having chronic gingivitis from both the sexes with age ranging from 18 to 55 years, 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. Patients were recalled after 1-month follow-up for evaluation of the clinical parameters only. The patients were randomly allotted using computer-generated random sequence table to one of the three groups by one examiner while the treatment was performed by another examiner. Three groups included in the study were as follows:

1. Group I: Chlorhexidine used as ultrasonic coolant (22 participants)

2. Group II: cardamom extract used as ultrasonic coolant (22 participants)

3. Group III: DW used as ultrasonic coolant (22 participants).

Cardamom extract preparation-

The entire procedure was performed under proper aseptic conditions .Fresh cardamom was taken from the botanical garden. It was ground to a fine powder in a mechanical grinder. Ten grams of this finely powdered cardamom was mixed with 100 ml of sterile deionized water and kept in a water bath in a round-bottomed flask at 55°C–60°C for 5 h and then filtered through sterile filter paper (Whatman®, UK). 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 DW to give a final concentration of 20% w/v.[4]

Clinical procedure

All treatment procedures were conducted in a closed operatory where fumigation facility was available. Before 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 to get rid of contaminated water due to overnight stagnation in waterlines. Thirty minutes before the procedure, a blood agar plate was positioned on the plate 1 spot for a period of 15 min. This was then subjected to microbial assessment in order to check for environmental contamination, if present, in the operatory. The procedure on the patients commenced only after the operator was assured that there is no environmental contamination seen on the agar plate. Sixty six patients who met the inclusion criteria were selected. The type of procedure to be performed was fully explained, and written informed consent was obtained from each patient. Patients were randomly allocated to one of the following three groups: chlorhexidine as ultrasonic coolant (Group

I), cardamom extract as ultrasonic coolant (Group II), and DW as ultrasonic coolant (Group III). Dental chairs with self-contained water system were selected for the study. The above-mentioned agents were added in the dental unit waterlines (DUWLs). Strict asepsis was observed inside the operatory, and the selected participants were prepared to enter the operatory by wearing headcaps and autoclaved gowns. The participants were instructed to refrain from all the actions that would generate aerosols.

Various actions such as conversation, sneezing, and coughing were strictly forbidden. Single-sitting ultrasonic scaling was done for all the patients for a period of 20 min, using ultrasonic scaler. During each scaling procedure, saliva ejector was used. After the completion of the procedure, the patients in each group were asked about any discomfort noticed such as alteration in taste or burning sensation during debridement procedure. Patients were asked to report to dental office if any adverse effects were experienced post treatment.

Position of agar plates

Blood agar was chosen because it is a general purpose, nonselective, and enriched medium that promotes the growth of microorganisms, such as those sampled from air. Position of the agar plates was chosen based on the findings from a previous study conducted by Yamada *et al.*[5] for reproducibility of the data. Figure 1 shows the three graphical 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, i.e., the mouth of the patient. One plates at each position for aerobic culture were placed on the patient's chest, right side, and left side, respectively [Table 4]. Two plates were deliberately placed to check whether amount of colonies formed were almost quantitatively identical.

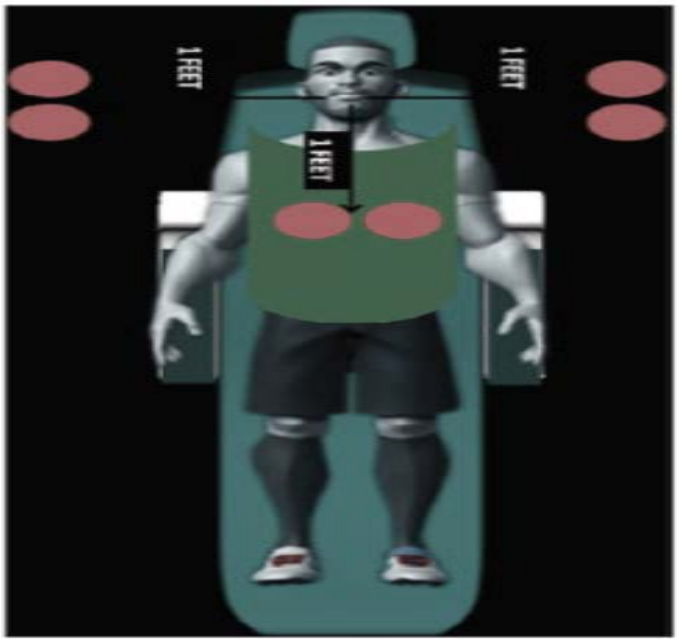

Figure 1: Schematic illustration of blood agar plates placed at three different location

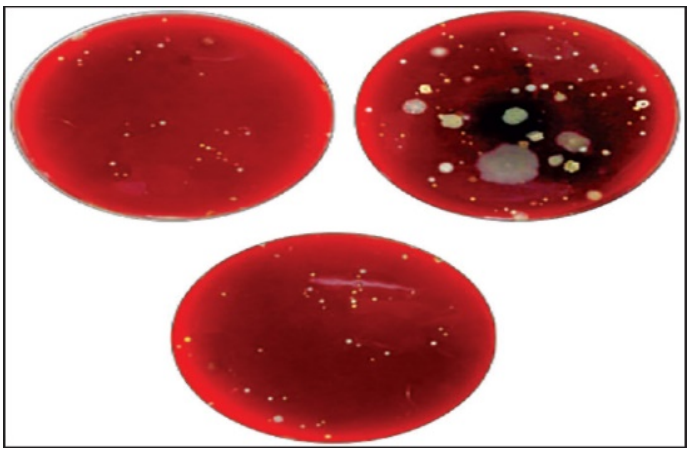

Figure 2: Colony-forming units of all three group on blood agar plates

Microbial analysis

Analysis of aerosols: The aerosols from the ultrasonic unit were collected on two blood agar plates placed at three different positions, each within a range of 1 foot, in all the three groups. After collecting the samples, plates from each position were incubated aerobically for 48 h.

Statistical analysis

Statistical analysis of the results was done for CFUs using Statistical package for social science. 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. Intergroup analysis of the clinical parameters (GI and PI) was also performed using ANOVA, whereas intragroup analysis was done using Student's *t*-test. The statistical significance was defined as $P < 0.05$.

Results

There was no significant difference within the groups with respect to demographic characteristics ($P > 0.05$). The mean ± SD age of the patients included in the study was 29.26 ± 2.86 years. A total of 58.33% males and 41.67% females participated in the study. No adverse effect was reported by the patients during or after 1-month follow-up [Figure 2].

Table : Demographic characteristics of the patients

Patients	Group I	Group II	Group III	*P*
Age	29.20±3.05	29.30±2.71	29.3±2.97	0.99*
Number of teeth	29.15±1.63	29.45±1.50	29.85±1.49	0.36*
Male/female	11/9	12/8	12/8	1.28*

Figure 2 shows the colonies formed on the blood agar plates for all the three experimental groups. Figure 3 graph shows the graph where illustration of mean ± SD of the CFUs formed is summarized. Table 4 shows the mean ± SD scores of CFUs of all the three groups. In Group I, mean ± SD scores of CFUs formed at the chest, right side, and left side of the patients were 627.35 ± 34.10, 407.6 ± 25.87, and 403.55 ± 16.93, respectively. In Group II, mean ± SD scores of CFUs formed at the chest, right side, and left side of the patients were 575.9 ± 30.41, 419.5 ± 48.21, and 413.5 ± 51.37, respectively. In Group III, mean ± SD scores of CFUs formed at the chest, right side, and left side of the patients were 1396.0 ± 214.93, 1064.05 ± 26.69, and 1009.85 ± 23.29 (mean ± SD), Respectively.

Table : Colony-forming units according to the different location of all the groups

Location of the plate	Group I	Group II	Group III	*P*
Patient's chest area	627.35±34.10	575.9±30.41	1396.15±214.93	<0.05†
Patient's right side	407.6±25.87	419.5±48.21	1064.05±26.69	<0.05†
Patient's left side	403.55±16.93	413.5±51.37	1009.85±23.29	<0.05†
P	<0.05†	<0.05†	<0.05†	

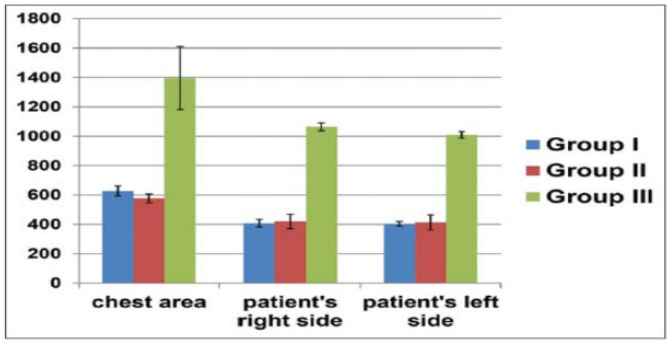

Figure : Graph illustrating the mean colony-forming units of all three groups

In the current study, it was found that CFU counts were highest on the blood agar plates placed at the patient's chest area followed by the patient's right side and left side, respectively.

Discussion

Aerosols generated during different dental procedures potentially spread the infection. Aerosols produced during dental procedures have the potential to spread infection in the dental office. According to the recommendation of the American Dental Association, potentially contaminated aerosols or splatter should be controlled during various dental procedures. Various studies have been conducted evaluating the effect of preprocedural mouthrinses on the dental aerosol contamination and have shown positive results. However, very few studies have been conducted evaluating the effect of antimicrobial mouthrinses used as ultrasonic coolant on the dental aerosols. Thus, the present study was conducted evaluating the effect of chlorhexidine and cardamom extract as an ultrasonic coolant as compared to DW on the reduction of microbial load in dental aerosols produced. In addition, their effect on the gingival status was also analyzed.

In the present study, antimicrobials as ultrasonic coolant were used instead of preprocedural mouthrinsing due to various reasons. Preprocedural mouthrinsing requires 30–60 s of rinsing period which varies according to various studies. Many studies have shown that 30-s period is enough to reduce bacterial count, but others suggest that

60-s rinsing period is required to produce any effect on bacterial count. This suggests that there is still no agreement among the researchers regarding the period of rinsing. On the other hand, using antimicrobial agents as an ultrasonic coolant provides continuous action of the agents over a longer period, thus bypassing the rinsing period as that of preprocedural mouthrinses. In addition, with the use of preprocedural mouthrinses, depth of penetration is less as compared to that of an ultrasonic coolant. Apart from these reasons, the patient's compliance and subjective error in rinsing was also the reason why ultrasonic antimicrobial coolants were chosen in the current study[6,7]

In the present study, chlorhexidine 0.2% and cardamom extract 20% w/v were used. Gupta and Jain 2015 evaluated the effect of chlorhexidine and cinnamon extract mouthrinses on gingival status and dental plaque levels. They found that the chlorhexidine group showed the maximum decrease in both plaque and gingival scores, followed by cinnamon extract, but the result was statistically insignificant. Based on this body of evidence, these two mouthrinses were used as ultrasonic coolant in the current study.24

In the present study, we found that chlorhexidine and cardamom were equally effective in improving the gingival status and reduction of plaque levels as compared to DW. However, the results of the present study are contradictory to a previous study conducted by Guarnelli *et al*. 2008 [8]where they found no clinical benefits with the use of chlorhexidine as an ultrasonic coolant over the water. However, one thing that needs to be taken in consideration is that the group patients included in our present study were those who were diagnosed with gingivitis as compared to aggressive periodontitis patients. This might have influenced the results of the present study as healing response for both the groups of patients is differs significantly. In the current study, it was found that CFU counts were highest on the blood agar plates placed at the patient's chest area followed by the patient's right side and left side, respectively. These results are in agreement with a previous study conducted by Gupta *et al*.[4] and Feres *et al*.[9] who also found that the highest CFU counts were seen on the blood agar plates placed at the patient's chest area.

A previous study conducted by Jawade *et al*.[10]where they found that chlorhexidine when used as ultrasonic coolant caused the highest reduction in CFU counts as compared to povidone-iodine and DW. Similar results were reported by Bay *et al*.(7) who found that there was no significant difference in the CFU counts between the patients rinsing with either chlorhexidine or essential oils. Chlorhexidine when used either as mouthrinses or as ultrasonic coolant has shown substantial results and thus been called as a gold standard in plaque control. An important finding from our study was that of cinnamon extract group, which showed comparable benefits as that of chlorhexidine. As reported in literature that chlorhexidine cannot be tolerated by all the patients due to its side effects such as staining of the tooth, dryness of mouth, and slight alteration in taste, cardamom extract mouthrinses can act as an alternative for the same, and this was proven in a previous study conducted by Gupta and Jain[11] where they found that cinnamon extract mouthrinses had almost similar effects on the gingival status as compared to chlorhexidine. They concluded that cinnamon might prove to be an effective agent owing to its ability to reduce plaque level and gingivitis.

In the present study, apart from evaluating the CFU counts on blood agar plates placed at a different location, CFUs from DUWLs were analyzed. It was found that least CFU counts were seen in cardamom extract group as compared to chlorhexidine and DW group. However, similar to

effect on aerosol, there was no statistical difference between the chlorhexidine group and cardamom extract group. DUWL analysis was done as they are known to harbour appreciable amounts of bacteria 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 professionals. Thus, it can be said that when antimicrobials are used as a coolant, it can also effectively reduce the bacterial counts in DUWLs although not totally able to eliminate them as suggested by the results of the present study.

While interpreting the results of the current study, its limitation should also be taken into considerations. In the present study, CFU counts of only aerobic bacteria were done. Viruses, anaerobic bacteria, fungi, and those requiring special media for its growth were not analyzed. Moreover, no attempt was made to avoid the fallout of viable bacteria at different positions, which might have underestimated the true extent of bacterial populations in air samples.

verall, the results of the present study demonstrate that cardamom extract was as effective as that of chlorhexidine when used as an ultrasonic coolant. To the best of our knowledge, this is the first study where cardamom extract as an ultrasonic coolant has been compared with that of chlorhexidine. Thus, more randomized controlled clinical trials with larger sample size need to conducted to validate these findings. Longer duration of evaluation will give results that are more predictable and would confirm its stability.

Conclusion

Within the limitations of this study, both cardamom and chlorhexidine when used as an ultrasonic coolant effectively helped in the reduction of bacterial contamination in dental aerosols which was seen by reduction in the CFUs, after adding these agents in the DUWL. Cardamom extract can also be promoted to be used as a mouthwash as it has no side effects. Moreover, its low cost may motivate the patients at especially low socioeconomic strata for oral hygiene maintenance. This is an encouraging result which clearly favors the promotion of cardamom among the rural communities, especially belonging to low socioeconomic strata, as cardamom is easily available, inexpensive, and a safe alternative to chlorhexidine. Furthermore, 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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