Comparative Efficacy of Eucalyptus Oil and Commercially Available Disinfectant Against Dental Unit Waterlines- An Experimental Study

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ABSTRACT

Introduction- Dental unit waterlines are heavily contaminated with microorganisms and pose a potential source of infection for a dentist as well as patients in dental settings.Disinfectants can be used to prevent the same. **Aim-** To compare the efficacy of eucalyptus oil and a commercially available disinfectant against dental unit waterlines **Materials and methods-** In the present study baseline water samples were obtained from the dental units after which they were treated with Continu disinfectant and eucalyptus oil in the concentration of 1:1, 1:10 and 1:100 for seven days and compared for a total viable count. **Results-** There was a reduction in mean CFU/ml when treated with disinfectants each for a period of 1 week.Eucalyptus oil 1:1 concentration was comparable with Continu disinfectant. **Conclusion-** The quality of water used in the dental procedures should be improved. It is found in the study that herbal disinfectants can be an option for chemical disinfectants available for treating microbial contamination of dental waterlines.

KEYWORDS: Disinfectant, Dental Unit, Eucalyptus, Microbial Load

INTRODUCTION

Dental unit waterlines (DUWLs) are an integral part of dental treatment systems. They provide water for lubrication during mechanical processes, instrument cooling and irrigation during treatment.[1] The growth of microbes in dental unit waterline biofilm are derived from the mains water line that supplies the dental unit. It takes approximately eight hours for a dental unit biofilm to form after water flows through it. Infection control in dentistry remains an area of major concern. Biofilm formation in dental unit waterlines takes place even when it is not used for the treatment of patients. The DUWLs property is such that they will develop a biofilm, and water passing the biofilm coated waterlines will contribute to increment in microbial load as it exits the tubing.[2] Recurrent periods of water stagnation in DUWLs and the properties of the plastics used in DUWLs production promotes the adherence and colonization of biofilm forming microorganisms.

Bacterial growth in the water sources is a growing problem as medical technology advances. Dental practice has no exemption and interest in the role of bacteria and microbes within dental units as a potential supply of cross infection is rising.Effective infection control is one of the cornerstones of good practice and clinical governance. Identifying all probable routes of transmission of infectious agents is a key constituent of any infection control practice. In dentistry of particular concern are those devices that are placed within the oral cavity and that are not easily or routinely disinfected because of their design or some other consideration.[3]

The ADA in 1996 recommended maximum permissible levels of <200 CFU/mL, and the Centre for Disease Control and Prevention (CDC) recommended<500 CFU/mL for non-surgical dental procedures. Direct source of DUWL bacterial infectivity are municipal water piped into the dental unit, the suck-back of patients' saliva during operative procedures, into the line due to lack of preventive valves, and contamination from free bottled water systems. An indirect source of contamination within the waterlines is a biofilm developing in the small-bore plastic tubing. Biofilms are microbial communities that adhere to solid surfaces wherever there is sufficient moisture and favorable environment for growth.[4] The water leaving the waterlines of dental units is frequently contaminated by the pathogenic and opportunistic micro-organisms.

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Detachment of micro-organisms or their DNA from dental unit biofilm flushed into the oral cavity could infect the patient.[5] Splatter and aerosols from contaminated water during dental procedures may possibly affect the patient and health care personnel.

There is irrefutable scientific evidence that DUWL provides the ideal environment for the development of microorganisms that enters dental units from the municipal water supply and from formerly-treated patients. Dental treatment can lead to transient bacteremia caused by oral streptococci. Escalating numbers of people with compromised resistance to overt and opportunistic pathogens, are seeking dental treatment. This population not only includes people with HIV infection, but also elderly people, organ transplant and blood transfusion recipients, and people with cancer, diabetes, and other chronic disorders.[6] Some of these people might be particularly susceptible to infection as a result of exposure to dental unit water.

To reduce the risk of infection both for the patients and for the health personnel, use of techniques such as waterline flushing, independent water reservoir systems, use of distilled water, ultraviolet light, inline micro porous filtration, and intermittent or continuous chemical disinfection has been recommended.[4] Biocides are available which are effective in reducing the bacterial load, but with the disadvantage of corrosion to the instruments used in the dental chair. Also it might be a cause of allergy to the patients as the waterlines are run through these biocides.

Eucalyptus, a native species from Australia, belongs to Myrtaceae family. It is one of the world's most important and most widely planted genera, mainly cultivated for its timber, pulp and essential oils that have medicinal property and therapeutic uses.[7] The value of Eucalyptus oil for medicinal purposes is based largely on the content of a particular oil constituent: 1,8-cineole.It is also known to contain bioactive products that showed antibacterial, antifungal , analgesic and anti-inflammatory effects , anti-oxidative and anti-radical activities.Since ages, essential oils as antimicrobial agents has been described qualitatively for many years and, in the specific case of oral microorganisms, essential oil mouthwashes have been demonstrated to be beneficial and safe. More research is required to know the spectrum of action of essential oils against oral microorganisms.[8]

Continu dental waterline disinfectant (Nu-view,UK) is a commercially available non-alcoholic disinfectant recently marketed containing the active ingredient quaternary ammonium compounds. It is a water based disinfectant that is safe for the patient and non-corrosive to dental instruments. The bactericidal action of the quaternary compounds has been attributed to the inactivation of energy producing enzymes, denaturation of essential cell proteins, and disruption of the cell membrane.[9]

Need of the study: Chemical disinfectants being a risk for infection and other side effects, this study was conducted to check the efficacy of natural products in disinfecting the dental waterlines.

Null Hypothesis: There is no difference in the efficacy of eucalyptus oil and non-alcoholic disinfectant in disinfecting dental waterlines.

Aim: To compare the efficacy of eucalyptus oil and commercially available non-alcoholic disinfectant in reducing microbial contamination of dental unit waterlines.

Objectives:

- To compare the efficacy of eucalyptus oil and commercially available disinfectant as disinfectants for dental unit waterlines.
- To assess the effectiveness different concentration of eucalyptus oil against dental unit waterlines.

MATERIALS AND METHODS

It is an experimental study and water samples of dental chairs from the clinical departments in K.M Shah Dental College were obtained. Ethical approval was obtained from the university for carrying out the study. A total sample size of 24 was obtained which achieves the power of 80% to detect the differences in the means among the groups. The alpha error was set at 5% and probability at 50%.

Preparation of the extract: The extract preparation was done in the pharmacological laboratory of College of Pharmacy, Sumandeep Vidyapeeth University. This study considers only the qualitative analysis of the activity of Eucalyptus leaves extract against the dental waterline biofilm. Therefore, the three concentrations, that is 1:1, 1:10 and 1:100 v/v was tested for antibacterial activity.

The plant materials were steam distilled for 90 min in full glass apparatus. The oils were isolated using a Clevenger-type apparatus. The extraction was carried out after 4 hour maceration in 500 ml of water. The oils were stored in dark glass bottles in a freezer until they were used. Further the oils extracted, was diluted in the ratio of 1:1, 1:10 and 1:100 with DMSO solution.[10]

Baseline water samples were obtained at the start of the study from two points i.e. from the outlet of a high speed hand piece and 3-way syringe. Before sample collection, the end of each hand piece and 3-way syringe was disinfected with 70% alcohol to avoid other sources of contamination. Water splashing was minimized during the filling of sample container and any kind of contact between the hand piece and the container was avoided. A volume of 10–50 ml of water was collected in sterile containers containing 0.1 gm 100mL^{-1}of sodiumthio-sulphate ($Na_2S_2O_3 \cdot 5H_2O$) to remove residual chlorine. Samples were stored in a refrigerator unitil investigation and processed at the laboratory withintwo hours.[1]

The two disinfectant samples A(Eucalyptus oils), B(Continu Disinfectant) were added to the reservoir bottle. The start of the study was at the end of clinical working hours at which 50mL of the disinfectant solution was added to the reservoir bottle that supplied the dental unit, and the solution was run through the system for about 2 minutes.The unit was turned off, and the disinfectant was left in situ overnight. Before the commencement of next day's work, water samples of 10–50mL from each one treated unit's air/water syringe were collected separately in sterile containers under aseptic conditions and labeled before treating the first patient of the day and quantified for total viable counts[4]. The reservoir bottles were again filled with water used on regular basis.

The above procedure was followed for 1 week, and final samples were obtained on the 7th day.

Microbial analysis: R2A agar(Hi-Media Laboratory Mumbai) was procured, and the microbial analysis was done in Department of Oral and Maxillofacial Pathology, K.M Shah Dental College. The water samples were vortexed to mix it properly. One loop (1/1000th ml of sample) was inoculated on the R2A Agar .The plates were incubated at 35˚C for 5 days. After 5 days the bacterial colonies were counted, and dilution factor was applied to obtain CFU/ml values.[10]

RESULTS

A total of 24 dental units were selected for the study, in which each intervention was given to 6 dental units for a period of 7 days. The water samples were collected at baseline and 7 days after usage of the disinfectant.

Table 1 shows the mean values for a reduction in bacterial count between the four interventional groups, where Group 1 was ContiNu waterline disinfectant, Group 2 was 1:1 concentration Eucalyptus oil, Group 3 was 1:10 concentration Eucalyptus oil and Group 4 was concentration Eucalyptus oil. On evaluation, statistically significant values was obtained for Group 1(*p=0.001*), Group2 (*p= 0.001*), Group3(*p=0.001*) and Group 4(*p=0.001*) .

Group		N	Mean ± SD	t value	P value
Group 1 – Continu	Before	6	326.66 ± 37.56	24.625	**.001 ***
	After 7 days		14.16 ± 7.62		
Group 2- 1:1 eucalyptus oil	Before	6	304.83 ± 13.00	23.755	**.001 ***
	After 7 days		16.66 ± 4.45		
Group 3 – 1:10 eucalyptus oil	Before	6	334.66 ± 52.90	14.058	**.001 ***
	After 7 days		63.16 ± 6.17		
Group 4 - 1:100 eucalyptus oil	Before	6	339.83 ± 42.06	15.820	**.001 ***
	After 7 days		110.83 ± 9.90		

*Statistically significant

Table 1: Comparison of mean bacterial count between Group 1 (ContiNu Disinfectant), Group 2(1:1 Eucalyptus oil), Group 3 (1:10 Eucalyptus oil) and Group 4 (1:100 Eucalyptus oil) after 7 days

Table 2 shows the correlation between the three concentrations of eucalyptus oils – 1:1, 1:10 and 1:100. Among all the three concentrations, the 1:1 ratio eucalyptus oil showed the highest correlation with a statistically significant value (p=0.010).

Group	N	Correlation	Significant
Pair 2 – 1:1 eucalyptus oil	6	0.917	**0.010***
Pair 3 – 1:10 eucalyptus oil	6	0.535	0.274
Pair 4 – 1:100 eucalyptus oil	6	0.733	0.098

*Statistically significant

Table 2: Correlation between the efficacy of three concentrations of eucalyptus oil

DISCUSSION

The incidence of adherent microbial biofilms in dental waterlines has been described for many years (Blake, 1963). The interest in these biofilms has been reawakened recently due to increasing number of immune-compromised dental patients and also due to an increase in awareness of occupational hazards in the dental offices (Costerton, Lewandowski & Caldwell, 1995).[11]

The internal surface of the tubing of dental units provides a constant reservoir of microorganisms. With the increase in the number of immune-compromised and medically compromised individuals receiving regular dental treatment, contaminated dental unit output water pose a severe risk of infection. The patients, dentists and auxillary staffs are usually at the danger of being infected with opportunistic pathogens such as *Pseudomonas* or *Legionella* species through cross infection or subsequent aerosol formation from water stemming from DUWLs.[1] Thus effective practical methods are needed for controlling the microbial contamination of DUWLs.

High concentration of water borne organisms in the DUWLs can cause multiple public health problems. Adulteration of waterlines could be inhibited by means of some disinfectants. Elimination of these substances from water and further delivering it into patient's mouth may reduce the potential for post treatment inflammatory episodes. The purpose of this study was to determine the extent of bacterial contamination of DUWLs in the dental units of various departments in K.M Shah Dental College and to compare the effect of eucalyptus based disinfectant and ContiNu dental waterline disinfectant in reducing the bacterial density. The water contained in the tubing is an ideal habitat for a host of microorganisms, leading to biofilm formation. The water travels through the tubing only for a few hours every working day and is supplemented with organics and gets contaminated during operation. This warrants the growth of bacteria in the system, especially at the surfaces leading to the establishment of a stable source of inoculum for sterile water.[12]

In this study, the baseline contamination level expressed as the mean CFU mL^{-1} without the use of disinfectants (> 10^2CFU mL^{-1}) was found to be higher than the recommended level by AD (< 200 CFU mL^{-1}).The mean bacterial count was >300CFU mL^{-1}. One possible

explanation can be, the dental units have never been disinfected since the day of use and the tubing of the chairs are old, leading to more bacterial growth.

The ideal properties of an agent required to treat DUWL include low toxicity, low cost, ease of treatment, compatibility with a wide range of materials, and broad spectrum antimicrobial efficacy, especially against biofilms. An ideal DUWL disinfecting process should (i) kill bacteria in the water phase, (ii) kill biofilm-embedded cells, (iii) remove biofilm from the surface (as a "killed" biofilm can be a source of endotoxin and also allows rapid recolonization of a newand viable biofilm,which may occlude the tubing), (iv) be easily performed and offer continuous protection, thereby eliminating the root cause of poor dental unit water quality, and (v) provide continued efficacy during periods of nonuse, such as overnight and weekends.The disinfectant exhibiting these attributes would be easier to explain to the patients and easier for practitioners to manage than the remedial treatment processes.[1] In view of these ideal properties of the disinfectant, herbal-based disinfectant like eucalyptus was introduced which has antimicrobial properties. Eucalyptus being a natural ingredient is said to be nontoxic and biodegradable.

In the present study, the three disinfectants selected were compatible with the DUWLs of the dental chair units used in the study. There was a substantial reduction in the mean CFU count when DUWLs were treated with disinfectants each for a period of one week. The results were in compliance with Pareek S. et al[1] who showed good results using aloe vera as an herbal disinfectant.

Eucalyptus is found to have antimicrobial and antifungal properties. It consists of essential oil of high concentrations of 1,8- cineole, α-pinene, globulol and pinacarvone with well-documented antimicrobial activity.[7] Essential oils are capable of affecting the biofilm formation. They significantly decrease the bacterial adhesion and affect bacterial viability in the biofilms.[13] The efficacy of eucalyptus oil as an antibacterial agent is shown to have a wide range of effectiveness against Gram-positive (Gram +ve) and Gram negative (Gram −ve) bacteria. The anti bacterial efficacy of eucalyptus is also in accordance with study conducted by Raho & Benali (2012)[8]where they checked for its effect on S.aureus and E.coli.

Chemical compounds such as chlorhexidinegluconate, chlorhexidine acetate, sodium hypochlorite, povidone iodine, and Listerine have been advocated for use in DUWL. These are all complex molecules with varying degree of toxicity. The conventional disinfectants like sodium hypochlorite proves to be a problem as they are highly corrosive and the taste and smell are unacceptable. Some of these materials also have toxic effects and might pose dangers or reactions in the human mouth.[14] Eucalyptus is suggested to be a potent antimicrobial agent as it was helpful in reducing the microbial contamination of dental unit waterlines. It was also observed in the present research that with increasing the concentration, the effect of disinfectant also increased.

Eucalyptus is suggested to be a potent antimicrobial agent as it was helpful in reducing the microbial contamination of dental unit waterlines. Eucalyptus at 1:1 concentration was having similar properties when compared with ContiNu disinfectant. It was also observed in the present research that with increasing the concentration, the effect of disinfectant also increased.

General Suggestions:

- Chemical disinfectants can be replaced by eucalyptus based disinfectants.
- Eucalyptus is evidenced to be a cost-effective disinfecting agent.
- Use of chemicals will lead to clogging and corrosion of dental tubing and airotors which can be prevented by using eucalyptus based disinfecting agent.

CONCLUSION

To date, there is minimal epidemiological evidence that microbial contamination fromDUWLs is a significant infection risk to patients and dental staff. However, the potential for infection does exist, and the effects of DUWL contamination requires further evaluation. Improving the quality of water from dental unit waterlines is of significant importance. Every effort should be made to eliminate not only planktonic bacteria but also the biofilm within the waterlines.Eucalyptus being a natural substitute and cost effective can be used for disinfectant without posing any risk to patients.

Further research is advocated to test the efficacy and shelf life of eucalyptus not only in disinfecting the dental unit water but also to be applied in other areas for use as hospital disinfecting solution.

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