

Self Prepared Herbal Mouthwash Used As Pre-procedural Rinse in Reducing Dental Aerosol: A Substitute to Chemical Mouthrinse: A Clinico-Microbiological Study

Research Article

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Abstract

Aim: To evaluate and compare the efficacy of pre-procedural mouth rinses in reducing microbial content of aerosol product during ultrasonic-scaling procedures by viable bacterial count. **Materials And Methods:** 5 patients were assigned in each group: A- Neem, B -CHX, C-Triphala, D - Control Group. In Group A, B, C -Patient were asked to rinse their mouth with 10 ml mouthwash for 30 seconds before SRP, of which A and C are self-prepared herbal mouthwashes i.e Triphala and Neem. Aerosol will be collected, cultured and incubated on blood agar plates at specified sites from operator. CFU will be counted and result will be assessed statistically. **Conclusion:** The study suggests that 10 ml of Neem Mouth rinse when used 10 minutes prior to ultrasonic scaling is more potent in reducing the aerosol contamination as compared to the Triphala mouth rinse and commercially available 0.2 % Chlorhexidine mouthrinse. Also the reduction in aerosol content was seen in Tray location when rinsed with CHX and aerosol reduction in Spitoon and Chest location while rinsing with Neem mouthrinse.

Key Words: Aerosols, Pre-procedural rinsing, Chlorhexidine, Herbal Mouthwashes, Colony forming units.

Introduction

The growth of infectious microorganisms within aerosols is identified as an important health risk linked with specific occupations. (1) In spite of the efforts made to bring down these health hazards, they are inevitable in the work place by nature of the profession. (2) Most of the procedures carried out in the mouth, leads in the formation of aerosol and splatters which are usually mixed with bacteria, fungi, protozoa and even blood borne viruses. (3) The terms "aerosol and splatter" in dental environment were put forward by Micik in their pioneering work on aerobiology. (4) The microbial aerosol per concentrations in dental treatment rooms were related more with scaling procedures and to a minor extent with cavity preparation. (5) These aerosols may be inhaled into the lungs and reach the alveoli, or they may come in contact with the skin or mucous membranes thus result into infection. As suggested by Harrel and Molinari, the three levels of shield in the minimization of aerosols are the usage of: Personal Protective barrier such as masks,

gloves and safety glasses, routine use of preprocedural rinse and use of high evacuation device. (6)

Chlorhexidine (CHX) is still considered the gold standard antimicrobial mouthwash because of its broad spectrum of the bacterial activity and prolonged substantivity. (7) But it also has some side effects, notably tooth staining, taste alteration, supragingival calculus formation and, less commonly, desquamation of the oral mucosa. (8) On the other hand, herbal mouth rinses with their natural ingredients offer a safe and effective option that should be made use of in the most favourable way. (9) Chlorhexidine was compared with *Triphala* and *Neem* mouth wash as a preprocedural rinse. *Triphala* is a well known powdered preparation being used in Ayurveda and it consists of equal parts of the *Embica Officinalis* (*Amalaki*), *Terminalia Chebula* (*Harritaki*) and *Terminalia Vibhitaki*. *Neem* exhibits anti-hyperglycemic, immunomodulatory, anti-inflammatory, anti-viral, anti-fungal, anti-bacterial activity. (10)

Hence, this study was aimed to evaluate the effectiveness of Chlorhexidine and Prepared Herbal mouth rinses on the reduction of aerosol contamination produced by ultrasonic scaler. **AIM:**

To evaluate and compare the efficacy of pre-procedural mouth rinses (*Neem*, *Triphala*, Chlorhexidine digluconate) in reducing microbial content of aerosol product during ultrasonic-scaling procedures by viable bacterial count.

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Objectives

- To evaluate bioaerosol production by counting colony forming units in test groups and control group at various sites from the patient undergoing Scaling and Root Planing Procedure.
- To compare and correlate the bioaerosol production in test groups and control group.

Materials and Methods

This study was conducted on patients who visited the Department of Periodontology in the SGT Dental College, Gurgaon. This study was done in collaboration with the Department of Ayurveda, Pharmacology and Microbiology, SGT University. Patients were informed about the study and their inclusion was purely voluntary.

Study population

Twenty patients with Chronic Periodontitis with the age range of 25-55 years were recruited in the study.

Selection criteria

Table 1: Selection Criteria

Inclusion criteria	Exclusion criteria
Participants having minimum of 20 permanent teeth	The presence of any systemic disease
Participants diagnosed with moderate-to-severe periodontitis.	Patients received antibiotics or nonsteroidal anti-inflammatory drugs in the past 9–11 weeks.
Systemically healthy patients	Oral prophylaxis within the past 3 months
Participants indicated for full-mouth scaling in single sitting.	Pregnant and lactating mothers And Smokers

Table 2: Ingredients of mouth rinse

Mouth rinse	Trade name	Ingredients
Triphala	-	<ul style="list-style-type: none"> ✓ Triphala powder, ✓ Mint oil, ✓ Aspartame powder ✓ Stevia ✓ Methyl paraben ✓ Propyl paraben ✓ Chloroform
Neem	-	<ul style="list-style-type: none"> ✓ Neem powder, ✓ Mint oil, ✓ Aspartame powder, ✓ Stevia ✓ Methyl paraben ✓ Propyl paraben ✓ Ethanol
Chlorhexidine	CLOHEX	<ul style="list-style-type: none"> ✓ CHX gluconate solution I.P. diluted to CHX gluconate 0.2% in aqueous base

Preparation fo Mouth rinses

Step 1: Preparation of Triphala And Neem Powder

Triphala powder-The churna was prepared as per the procedure given in Ayurvedic department. All the

given ingredients were powdered separately and then mixed together in specified proportions and to get uniformly blended churna .

Neem powder-The fresh neem leaves was sundried for 2 days until all the moisture from leaves get dried and crispy .Then the dried leaves were put into a blender and was grinded into a fine powder.

Step 2: Extraction

To 150g of Triphala Powder, 500ml of distilled water and 1ml of chloroform was added.

To 150 g of Neem Powder, 50% of water and 50% of ethanol was added i.e 250 ml water and 250ml ethanol.

Step 3: Filtration

The mixture is then kept for 2 days for effective maceration and complete extraction.

- The macerated content was filtered through Muslin Cloth (Double Layered)
- The pre-filtered macerate was again processed through vaccum filter for the removal of particulate matter.

The obtained products were labeled as –

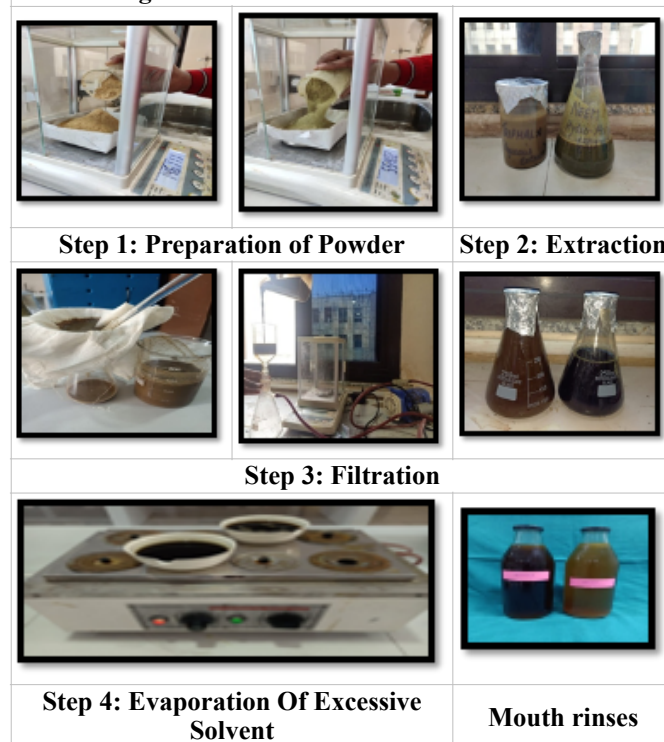
- A. Neem extract
- B. Triphala Extract

Step 4: Evaporation Of Excessive Solvent

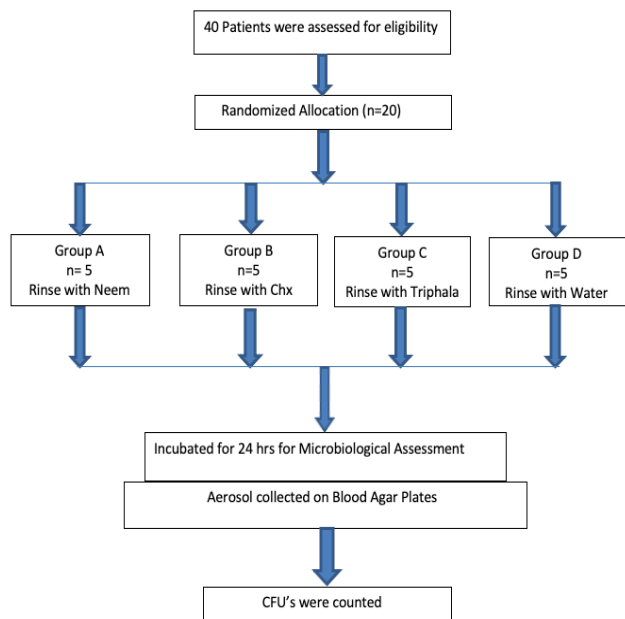
In order to remove the excessive amount of solvent the extracts were subjected to evaporation on water bath for 60 degree celsius.

The obtained product was the concentrated extracts for formulation of mouth rinses.

Figure 1: Lab Formulated Mouth rinses



Study design



Clinical procedure

Mouth Rinsing

In Group A and C -Patients were asked to rinse their mouth with 10 ml of prepared mouthwash and group B- with commercially available mouthwash chlorhexidine for 30 seconds before scaling and root planing procedure.

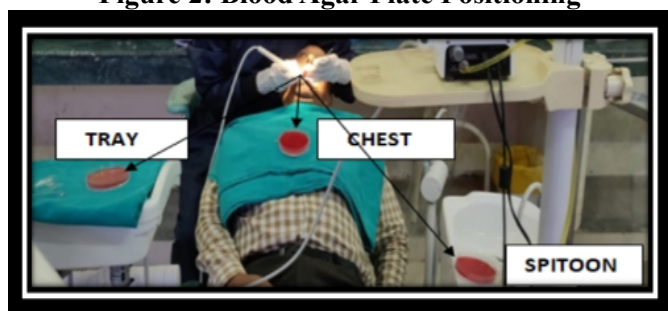
Test Groups

- Group A - Neem,
- Group B - Chlorhexidine digluconate
- Group C - Triphala

Control group

- Group D –Water

Figure 2: Blood Agar Plate Positioning



Blood Agar Plate Positions

- Reference Point: Patient's Mouth
- Plate 1- From Reference point to Chest - 12 inches
- Plate 2- From Reference point to Tray - 25 inches
- Plate 3 –From Reference point to Spittoon - 30 inches

Scaling and Root Planing Procedure

The scaling is done for 10 minutes and the aerosols are collected on the the blood agar plates which is positioned at specific sites(i.e placed at Tray, Chest, Spittoon) from the reference point (i.e Patient's Mouth) during scaling and root planing procedures.

Collection of Aerosols on Blood Agar plates

Aerosol which was collected, cultured on blood agar plates at specified sites from reference point and then Colony forming Unit was counted.

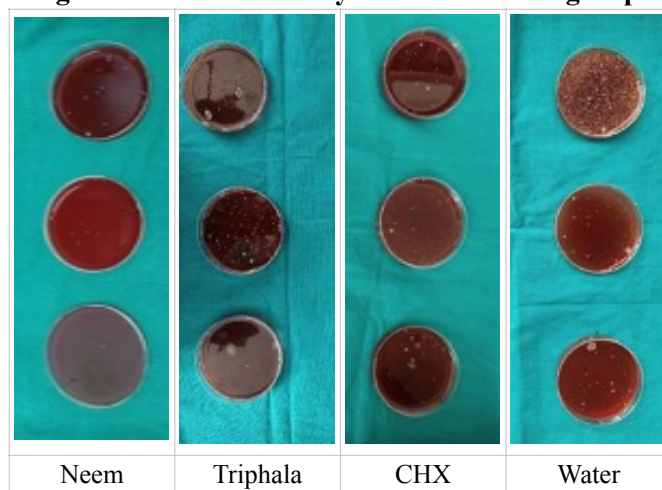
Microbial Analysis

After collecting aerosols on Blood agar culture plate. The plates were placed in an incubator and incubated at 37 degree Celsius for 24 hrs.

Figure 3: Incubator



Figure 4: Microbial analysis of all the four groups



Statistical analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 21, IBM Inc. Descriptive data was reported for each variable. Descriptive statistics such as mean and standard deviation for continuous variables was calculated.

Summarized data was presented using Tables and Graphs. Shapiro Wilk test was used to check the normality of the data. As the data was found to be normally distributed bivariate analyses was performed using One way ANOVA followed by tukey's for post hoc comparison. Level of statistical significance was set at p-value less than 0.05.

Analysis of variance

Analysis of variance (ANOVA) is used when we compare more than two groups simultaneously. The purpose of one-way ANOVA is to find out whether data from several groups have a common mean. That is, to determine whether the groups are actually different in the measured characteristic. One way ANOVA is a simple special case of the linear model. For more than

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two independent groups, simple parametric ANOVA is used when variables under consideration follows Continuous exercise group distribution and groups variances are homogeneous otherwise non parametric alternative Kruskal-Wallis (H) ANOVA by ranks is used. The one way ANOVA form of the model is

$$Y_{ij} = \alpha_j + \epsilon_{ij}$$

where:

- Y_{ij} is a matrix of observations in which each column represents a different group.
- α_j is a matrix whose columns are the group means (the “dot j” notation means that α applies to all rows of the j^{th} column i.e. the value α_{ij} is the same for all i).
- ϵ_{ij} is a matrix of random disturbances.

The model posits that the columns of Y are a constant plus a random disturbance. We want to know if the constants are all the same.

Assumptions are

- Response variable must be normally distributed (or approximately normally distributed).
- Samples are independent.
- Variances of populations are equal.
- The sample is a simple random sample (SRS).

Two-way ANOVA is used when we have one measurement variable and two nominal variables, and each value of one nominal variable is found in combination with each value of the other nominal variable. It tests three null hypotheses: that the means of

the measurement variable are equal for different values of the first nominal variable; that the means are equal for different values of the second nominal variable; and that there is no interaction (the effects of one nominal variable don't depend on the value of the other nominal variable). When we have a quantitative continuous outcome and two categorical explanatory variables, we may consider two kinds of relationship between two categorical variables, In this relationship we can distinguish effect of one factor from that of the other factor. This type of model is called a main effect model or no interaction model.

Tukey Multiple Comparison Test

After performing ANOVA, Tukey HSD (honestly significant difference) post hoc test is generally used to calculate differences between group means as

$$q = \frac{\bar{X}_1 - \bar{X}_2}{SE} \quad SE = \sqrt{\frac{S^2}{2} \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}$$

S^2 is the error mean square from the analysis of variance and n_1 and n_2 are number of data in group 1 and 2 respectively.

Statistical significance

Level of significance "p" is level of significance signifies as below:

- $p > 0.05$ Not significant (ns)
- $p \leq 0.05$ significant (*)

Results

Table 1 :Intergroup comparison of bioaerosol production by counting colony forming units in spittoon

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A- Neem	5	9.800	5.8052	2.5962	2.592	17.008	1.0	15.0
Group B-Chx	5	25.400	26.3591	11.7881	-7.329	58.129	4.0	70.0
Group C-Triphala	5	73.200	80.4189	35.9644	-26.653	173.053	4.0	200.0
Group D-Water	5	95.000	75.8288	33.9116	0.846	189.154	25.0	200.0
P value	0.100							

Table 1- Intergroup comparison of bioaerosol production by counting colony forming units in spittoon. Though colony count was found to be maximum in Group D and least in Group A followed by Group B and C, but this difference failed to reach the level of statistical significance as $p > 0.05$.

Table 2 -Intergroup comparison of bioaerosol production by counting colony forming units on chest

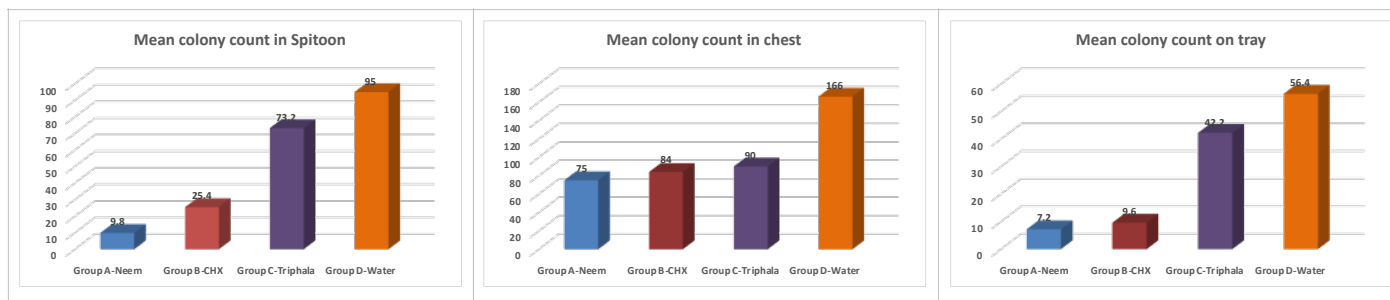
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A-Neem	5	75.000	45.5522	20.3715	18.440	131.560	30.0	150.0
Group B-CHX	5	84.000	14.7479	6.5955	65.688	102.312	70.0	100.0
Group C-Triphala	5	90.000	22.3607	10.0000	62.236	117.764	50.0	100.0
Group D-Water	5	166.000	47.7493	21.3542	106.711	225.289	100.0	200.0
P value	0.003							
Post hoc	4>3>2>1							

Overall significant difference was seen in the mean colony count when compared using one way ANOVA test as $p < 0.05$. Post hoc comparison using Tukey’s test showed significantly lesser colony count followed by CHX, Triphala and water group.

Table 3 - Intergroup comparison of bioaerosol production by counting colony forming units on tray

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group A-Neem	5	7.200	4.2778	1.9131	4.288	14.912	4.0	15.0
Group B-CHX	5	9.600	3.7014	1.6553	2.604	11.796	3.0	12.0
Group C-Triphala	5	42.200	60.8498	27.2129	-33.355	117.755	5.0	150.0
Group D-Water	5	56.400	80.2795	35.9021	-43.280	156.080	20.0	200.0
P value					0.354			

Table 3 –States that the Intergroup comparison of bioaerosol production by counting colony forming units on tray. Though colony count was found to be maximum in Group D and least in Group A followed by Group B and C, but this difference failed to reach the level of statistical significance as $p>0.05$.



Discussion

The generated aerosol while performing a dental procedure in concurrence with bacterial infection can cause a potential hazard to the dentist as well as to the patients. This study has confirmed that aerosol and splatter are produced in significant amounts by the ultrasonic scaler device in the arena of dentistry. There is a potential threat for infection to spread owing to the aerosols produced. This demands the use of mouth rinses before the procedure to possess slight bacterial load.(5)

In a study conducted by Fine et al, (11) it was shown that pre-procedural oral rinsing with an antiseptic mouthwash considerably decreased the viable microbial content of bio-aerosols produced during dental trials. It was recommended that this pre-procedural rinsing may have a significant part in reducing the risk of cross contamination with infectious agents in the dental operator. Gunjan gupta et al (12) compared the efficacy of 0.2% chlorhexidine, and herbal mouth wash(Test Groups) to water (Control group) and found that both the test groups reduced CFUs significantly when compared to the control group. They concluded that chlorhexidine group was superior to herbal mouth wash group. But in a study done by Koduganti Rekha Rani there were no statistical difference between the test groups i.e., chlorhexidine and herbal mouth rinse (13)

In the present study, the effectiveness of pre-procedural rinsing with herbal rinse was compared with 0.2% Chlorhexidine which was considered as a gold standard. The outcomes of this study revealed that 10 ml of Neem Mouth rinse when used 10 minutes prior to ultrasonic scaling is more effective in decreasing the aerosol infection as compared to the Triphala mouth rinse and commercially available 0.2 % Chlorhexidine mouthrinse. And also the decrease in aerosol content was seen in Tray location when rinsed with CHX and

aerosol reduction in Spitoon and Chest location while rinsing with Neem mouthrinse. Also the patient’s chest location was more exposed to the microbial aerosols as compared to the tray location and spitoon location which demands the usage of preventive methods to lessen cross contamination in a dental practice.

Even though the discovery of chlorhexidine was done in the early 1950s, it is still well thought-out as the most effective antiplaque agent in dentistry. However, because of its displeasing taste and proclivity to stain the teeth brown, its use is limited. As the taste should not be a hindrance for its use with maximal inhibition of bacteria and plaque, 2% of neem was used in this study. For reducing periodontal that registers as chlorhexidine, neem mouthwash was very effective. When compared with 0.2% chlorhexidine gluconate mouthwash, neem mouthwash is considered to be costeffective. Hence, the neem extract can be used as a healthier substitute mouthwash to 0.2% chlorhexidine gluconate mouthwash in low socioeconomic status population. Chlorhexidine mouthwash revealed a greater effect on the reformation of plaque and inflammation of gingiva when compared with that by neem mouthwash.(14)

Though aerosol production cannot be completely eradicated with infection control procedures, the putative potential of these aerosols can be minimised by preprocedural rinsing. Pre-rinsing with herbal mouth rinse was more effective than chlorhexidine in this study which promotes the use of herbal rinses in the dental setting.(13)

Conclusion

The study suggests that 10 ml of Neem Mouth rinse when used 10 minutes prior to ultrasonic scaling is more potent in reducing the aerosol contamination as compared to the Triphala mouth rinse and commercially available 0.2 % Chlorhexidine mouthrinse. And also the

reduction in aerosol content was seen in Tray location when rinsed with CHX and aerosol reduction in Spittoon and Chest location while rinsing with Neem mouthrinse.

It can be concluded as: Neem > Chlorhexidine > Triphala > Water

Also the patient's chest location was more exposed to the microbial aerosols as compared to the tray location and spittoon location which necessitates the usage of preventive methods to reduce cross contamination in a dental practice.

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