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Comparison ofthe Efficacy of Preprocedural Mouthrinsing withA Triphala Mouthwash versus A Chlorhexidine Mouthwash on Aerosol Contamination Produced By Ultrasonic Scaling – A Randomised Controlled Trial

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Abstract
*Dentist, dental hygienistand oral health care workers practice in a highly contaminated environment and get exposed to variety of bacteria, viruses, fungi and protozoa from many sources.One of the methods of reducing microbial load is preprocedural rinsing.Aim:To compare the efficacy of preprocedural mouthrinsing with triphala mouthwash and chlorhexidine (CHX)mouthwashon aerosol contamination produced by ultrasonicscaling. The 50 subjects were randomly divided intotwogroups. The subjects wereadministered 10ml of mouthwash 10 minutes prior to theprocedure and were asked to rinse for 1 minute.Results:**On intergroup comparison of the mean colony forming units (**CFUs)**at doctor's chest area,patient'schest area and assistant's chest area for GroupA(Triphala)and GroupB(CHX),**GroupB showed greater reduction of CFUs at all the chest areas.**Onintragroup comparison** of the number of CFUs,higher mean CFUs seen in patient's chest area followed by doctor's chest areaand least for assistant's chest area.Conclusion: 10 ml 0.2% Chlorhexidinewhen used for 10minutes prior to ultrasonicscaling is more potent in reducing the aerosol contamination than that with 10 ml 0.6% triphala mouthwashand patient's chest area was more exposed to the microbial aerosols.*

***Keywords**: Aerosols, preprocedural mouthwash,triphala,chlorhexidine,blood agar plate*

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I. Introduction

The spread of infection through **aerosol and splatter** has long been considered one of the main concerns in the dental community because of their potential harmful effects on the health of doctors, patients and dental personnel.[1] The smaller particles of an aerosol (0.5 to 10 μm in diameter) have the potential to penetrate and lodge in the smaller passages of the lungs and are thought to carry the greatest potential for transmitting infections.[2] The oral cavity is a unique environment which can provide an ideal medium for bacterial growth.[3] Most of the dental procedures have the potential for creating contaminated aerosols and the splatter produced during dental operative procedures cause an increased risk of cross infection.[4]

In dentistry, the ultrasonic scaler is considered to be the greatest producer of small particle aerosol contamination.[4] In the dental clinic, dentists and patients are daily exposed to a great variety of infectious agents transported by aerosols and droplets.[4] The association of these aerosols with the respiratory infections, opthalamic and skin infections, tuberculosis and hepatitis B have been reported.[5] These aerosols maybe inhaled into the lungs to reach the alveoli or may come in contact with the skin or mucous membranes.[6] There are at least four potential sources of airborne contamination during dental treatment: dental instrumentation, saliva, respiratory sources, and the operative site.[7] Methods of reducing airborne contamination are preprocedural mouthrinsing, barrier protection such as masks, gloves and eye protections, rubber dams, and high-volume evacuators.[7, 8]

Chlorhexidine gluconate is considered as the gold standard of antimicrobial rinses because of its broad spectrum antibacterial activity and substantivity of 8 to 12 hours.[1] Chlorhexidine is an antimicrobial agent.[9] It acts on the inner cytoplasmic membrane hence it is a membrane active type of substance.[9] It is dicationic at pH levels above 3.5.[10] It prevents plaque accumulation, hence it is an antiplaque and antigingivitis agent.[9] However,unwanted side effects, such as temporary loss of taste; staining of teeth, restorations, and

mucosa; dryness and soreness of mucosa; bitter taste; and a slight increase in supragingival calculus formation are the limiting factors in extended use of this chemical plaque control agent.[1,11]

In the emerging era of pharmaceuticals, herbal medicines with their naturally occurring active ingredients offer a gentle and enduring way for restoration of health by the most trustworthy and least harmful method.[1] Herbal medicine is both promotive and preventive in its approach because it may be purchased over-the-counter and have attracted millions of consumers who are looking for an alternative mouthrinse.[1] The main benefit of using herbal preparations is that they do not contain alcohol or sugar, which are present in other over-the-counter products and which possess the ill-effects of causing bacterial growth resulting in halitosis or bad breath.[6]

Triphala is derived from Sanskrit words "tri" meaning three and "phala" meaning fruit.[12] Triphala churna is a synergy of three fruits : Amalaki (*Embilica officinalis)* , Bibhitaki (*Terminalis bellerica*) and Haritaki (*Terminalis chebula*).[12,13] Main constituents of Triphala are tannins, quinones, gallic acid, vitamin C, flavones, flavonoids and flavonols.[14] The ingredients of Triphala acts as an antiseptic, astringent, antihelmintic, hemostatic, expectorant, laxative, nutritive tonic and rejuvenative tonic.[15] The **usefulness of Triphala** is due to its antibacterial, antiseptic, anti-inflammatory, antimicrobial, antiplaque, analgesic, antipyretic, antiulcerogenic, wound healing, collagenase inhibiting (anti-collagenase) properties and its free radical scavenging property.[12,15] It can also be used as a mouthrinse, a denture cleanser, a root canal irrigant, and an anticancer, an anticaries and an antifungal agent.[12, 15] Other uses of Triphala are that, it acts as a natural laxative, colon cleanser and possible colitis helper.[12,15] It also helps in weight loss, lowering cholesterol, immunomodulation and diabetic management.[12,15] It also has some anti-arthritic properties.[12,15]Multiple beneficial effects of triphala were found to be effective in treating gingival and periodontitis diseases as well as equally effective to the gold standard mouthwash chlorhexidine in reducing the bacterial load when used as an preprocedural mouthwash.

Hence, **the present study** was conducted **to compare the efficacy of preprocedural mouthrinsing with a triphala mouthwash versus a chlorhexidine mouthwash in reducing the microbial load after ultrasonic scaling.**

II. Materials And Method

The study was conducted by selecting subjects from the Out Patient Department of Periodontology of Y.M.T. Dental College, Kharghar, Navi Mumbai. The study was a randomized controlled clinical trial.Patients were recruited for the study from August 2017 to August 2019. A total of 50 subjects, of either sex, within the age group of 30-65 years, having chronic periodontitis were included in the study. This study was conducted after clearance from the ethical committee of the institute was obtained. It was conducted on subjects who gave verbal and signed written consent after being informed about the study protocol in the language that was best understood by them. The subjects were selected on the basis of the following criteria :

INCLUSION CRITERIA

- Subjects with chronic periodontitis having probing pocket depth (PPD) of ≥ 4mm in four or more sites.
- Subjects within the age group of 30-65 years of either sex.
- Subjects with minimum of 20 teeth present in the dentition.
- Systemically healthy and cooperative subjects.
- Mean plaque score > 1 according to plaque index (PI) **(Silness and Loe, 1964)**[16].

EXCLUSION CRITERIA

- Subjects who had undergone any periodontal treatment in the past 3 months.
- Subjects with a history of antibiotic intake within the past 3 months.
- Pregnant or lactating women.
- Subjects with multiple carious lesions requiring immediate restorative treatment.
- Subjects with a history of oral prophylaxis within the past 6 months.

Subjects satisfying the inclusion and exclusion criteria were selected for the study. The clinical parameters plaque index (PI) and probing pocket depth (PPD) were recorded. The probing depth was measured using a UNC-15 graduated periodontal probe. Once the case histories of 50 subjects were taken, the subjects were randomly divided by simple randomization technique using lottery method into two groups

In GROUP A (25 subjects) – Triphala mouthwash group

10 ml of the preprocedural 0.6% triphala mouthwash was administered 10 minutes prior to the procedure. Subjects were asked to rinse with the assigned mouthwash for 1 minute.(Fig. 1)

In GROUP B (25 subjects) – Chlorhexidine mouthwash(Hexidine®) group
10 ml of the preprocedural 0.2% chlorhexidine mouthwash was administered 10 minutes prior to the procedure. Subjects were asked to rinse with the assigned mouthwash for 1 minute. (Fig. 2)

Fig. 1- Group A - 0.6% Triphala mouthwash

Fig. 2- Group B - 0.2% Chlorhexidine mouthwash

METHOD OF COLLECTION OF SAMPLE

All patients were posted as the first patient of the day in order to avoid cross-contamination. **Three** standardized locations of the operatory were chosen for evaluation in each treatment group - **doctor's chest area, patient's chest area and assistant's chest area**. (Fig. 3)

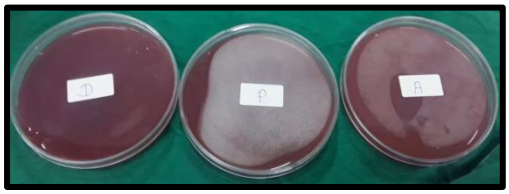
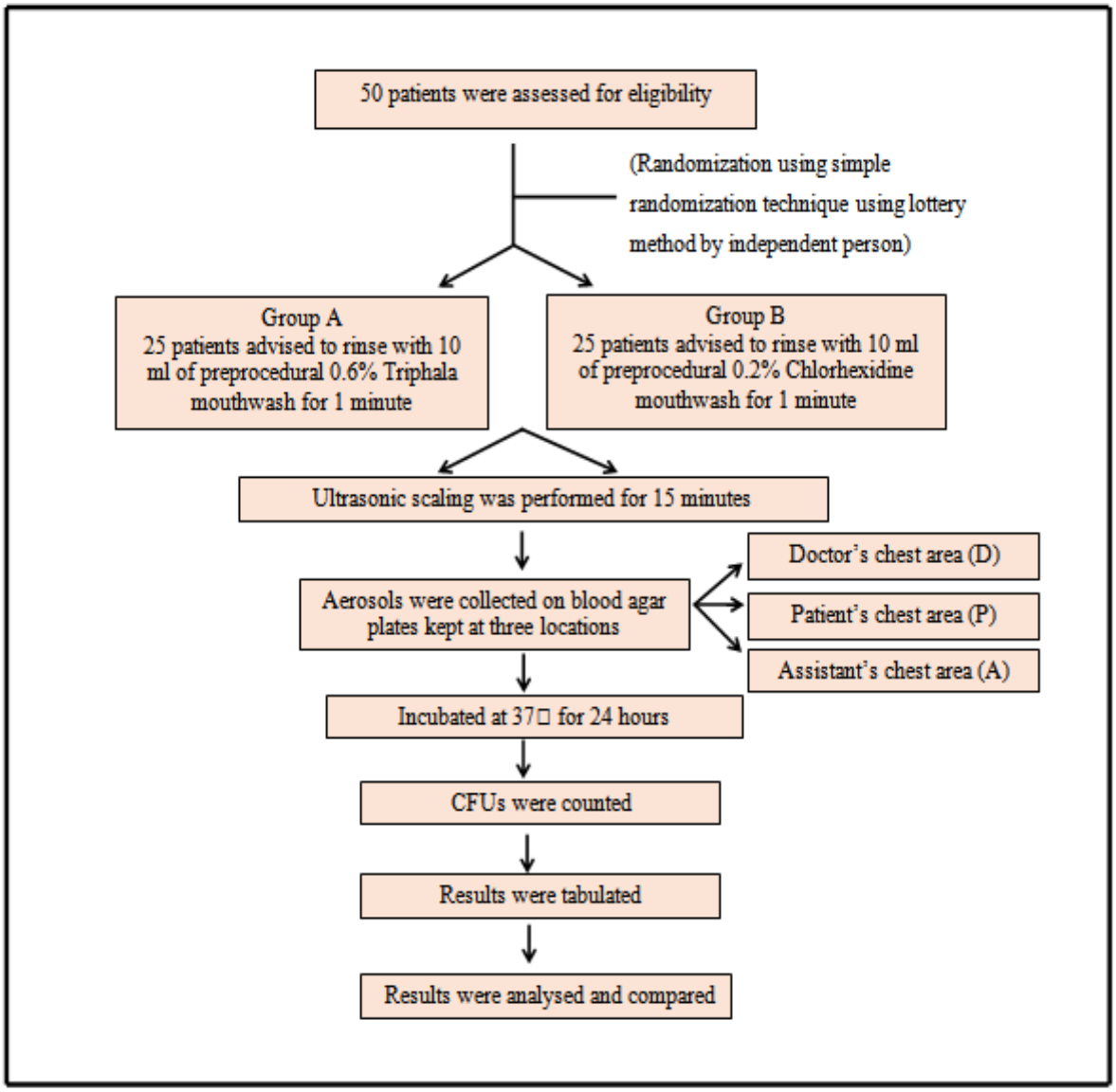


Fig. 3- Blood Agar Plates (labeled as D, P and A for doctor's chest area, patient's chest area and assistant's chest area))respectively)

Blood agar plates were used to collect the airborne microorganisms. Three blood agar plates labelled as D, P and A were placed at the three locations respectively. The average distance was 12 inches from the patient's mouth to the agar plate. Subsequently, 15 minutes of ultrasonic scaling was performed in both the groups. After collecting the samples, the blood agar plates were incubated at 37℃ for 24 hours. The number of colony forming units (CFUs) that grow at the end of 24 hours on each blood agar plate were counted. The study design is summarized in the following fig 4.

Fig. 4Flow chart of study design



STATISTICAL ANALYSIS

Data obtained was compiled on a MS Office Excel Sheet (v 2010, Microsoft Redmond Campus, Redmond, Washington, United States). Data was subjected to statistical analysis using Statistical Package for Social Sciences (SPSS v 21.0, IBM). Normality of numerical data was checked using Shapiro-Wilk test & was found that the data did not follow a normal curve; also data on CFU is numerical discrete, hence **non-parametric tests** have been used for comparisons. Inter group comparison (2 groups) was done using Mann Whitney U test. Intra group comparison (>2 groups) was done using Kruskal Wallis ANOVA followed by pair wise comparison using Mann Whitney U test. For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

III. Results

The mean age of the subjects in Group A (Triphala) and Group B (Chlorhexidine) was 39.60 years and 39.52 years respectively. **(Table 1, Graph 1)** Out of 50 subjects selected for the study,females accounted for 54% and males accounted for 46% of the total participants. **(Table 2, Graph 2) On inter group comparison between Group A (Triphala) and Group B (Chlorhexidine) with regard to mean Plaque index and Probing Pocket depth at baseline,** the mean plaque index was 2.5320 and 2.5012 and the mean probing pocket depth was 5.56 and 5.52for the two groups respectively. There was a **statistically non-significant difference** seen for the values between the groups ($p>0.05$) for plaque index**(Table 3, Graph 3) and** probing pocket depth **at baseline**. **(Table 4, Graph 4)**

At the end of 24 hours, on inter group comparison of mean CFUs at doctor's chest area for Group A (Triphala) and Group B (Chlorhexidine), the mean CFU at **doctor's chest area** was 29.88 and 21.32**(Table 5, Graph 5)**, at **patient's chest area** was 40.24 and 29.24 **(Table 6, Graph 6)** and at **assistant'schest area** was 22.96 and 15.04 **(Table 7, Graph 7)** for the two groups respectively, which means that there were higher values of mean CFU for group A (Triphala) as compared to group B (Chlorhexidine).Thus the analysis revealed that Group B (Chlorhexidine) showed a greater reduction of CFUs at doctor's, **patient's** as well as **assistant's** chest area as compared to Group A (Triphala). There was a **statistically highly significant difference** seen for the values between the groups ($p<0.01$) for means of CFUs at **doctor's,** at**patient's chest area** and at **assistant'schest area**.

At the end of 24 hours, onintra group comparison of the number of CFUs at Doctor vs Patient vs Assistant chest area in **Group A (Triphala)**, higher mean CFUs seen in patient's chest area (40.24) followed by doctor's chest area (29.88) and least for assistant's chest area (22.96). In **Group B (Chlorhexidine)**,higher mean CFUs seen in patient's chest area (29.24) followed by doctor's chest area (21.32) and least for assistant's chest area (15.04). There was a **statistically highly significant difference** seen for the values between different chest areas i.e. doctor's chest area, patient's chest area and assistant's chest area ($p<0.01$) for both the groups i.e. Group A (Triphala) and Group B (Chlorhexidine). (**Table 8(a), Graph 8)**

Also on **pairwise comparison** in **Group A (Triphala)**, there was a **statistically highly significant difference** seen for the values between doctor's chest area vs patient's chest area and patient's chest area vs assistant's chest area ($p<0.01$) and **statistically significant difference** seen for the values between doctor's chest area vs assistant's chest area ($p<0.05$). In **Group B (Chlorhexidine)**, there was a **statistically highly significant difference** seen for the values between doctor's vs patient's chest area, doctor's chest area vs assistant's chest area and patient's chest area vs assistant's chest area ($p<0.01$). (**Table 8(b), Graph 8)**

Table 1: Mean age of the subjects in Group A (Triphala) and Group B (Chlorhexidine)

Groups	N	Mean age	Standard Deviation
Group A	25	39.60	3.122
Group B	25	39.52	2.400

Graph 1: Mean age of the subjects in Group A (Triphala) and Group B (Chlorhexidine)

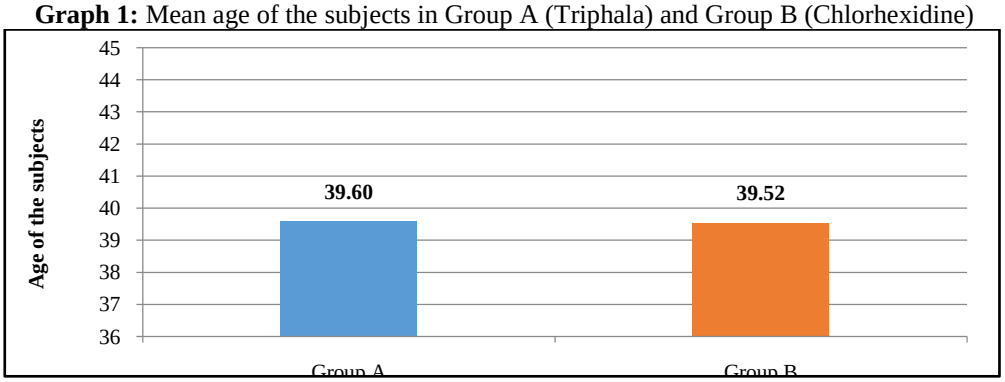


Table 2: Distribution as per gender in Group A (Triphala) and Group B (Chlorhexidine)

Gender	GROUPS			
	A	B	Total	Percent
Female	15	12	27	54 %
Male	10	13	23	46 %
Total	25	25	50	100 %

Graph 2: Distribution as per gender in Group A (Triphala) and Group B (Chlorhexidine)

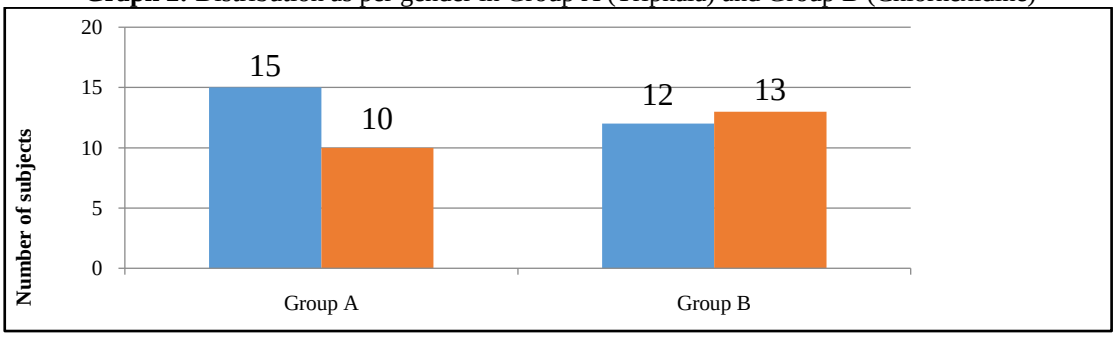


Table 3: Inter group comparison between Group A (Triphala) and Group B (Chlorhexidine) with regard to mean Plaque index at baseline

Groups	N	Mean PI	Standard Deviation	Median	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Group A	25	2.532000	0.2097419	2.56	280.5	-0.622	0.534#
Group B	25	2.501200	0.1400095	2.51			

Graph 3: Inter group comparison between Group A (Triphala) and Group B (Chlorhexidine) with regard to mean Plaque index at baseline

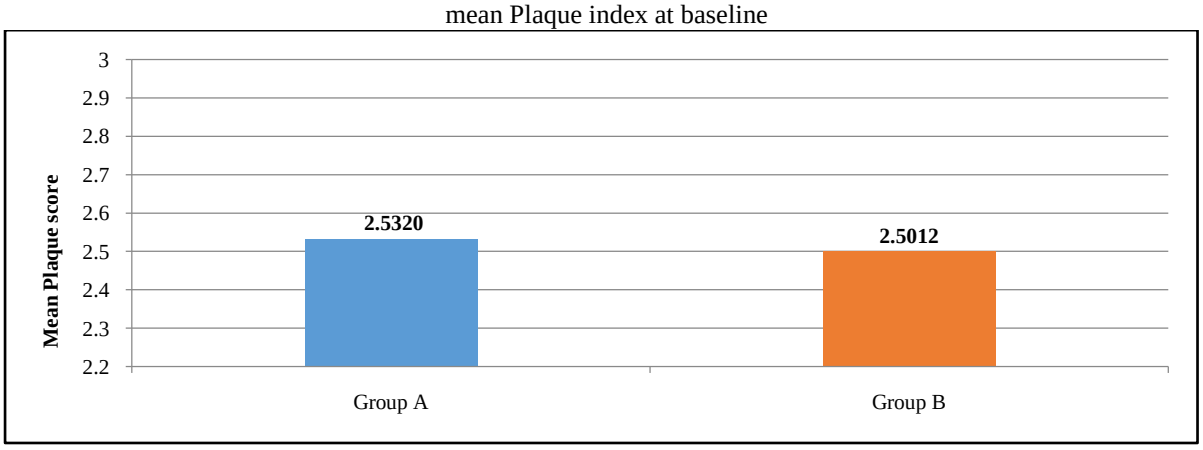


Table 4: Inter group comparison between Group A (Triphala) and Group B (Chlorhexidine) with regard to mean Probing Pocket depth at baseline

Groups	N	Mean PPD	Standard Deviation	Median	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Group A	25	5.56	0.507	6	300	-0.281	0.779#
Group B	25	5.52	0.510	6			

Graph 4: Inter group comparison between Group A (Triphala) and Group B (Chlorhexidine) with regard to mean Probing Pocket depth at baseline

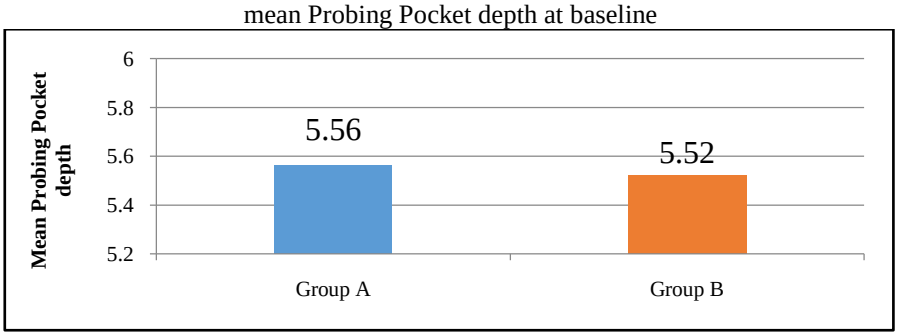


Table 5: Inter group comparison of mean CFUs at doctor's chest area for Group A (Triphala) and Group B (Chlorhexidine) at the end of 24 hours

Groups	N	Total no. of CFUs at doctor's chest area	Mean	StandardDeviation	Median	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Group A	25	747	29.88	9.567	30	147.5	-3.206	0.001**
Group B	25	533	21.32	6.902	20			

Graph 5: Inter group comparison of mean CFUs at doctor's chest area for Group A (Triphala) and Group B (Chlorhexidine) at the end of 24 hours

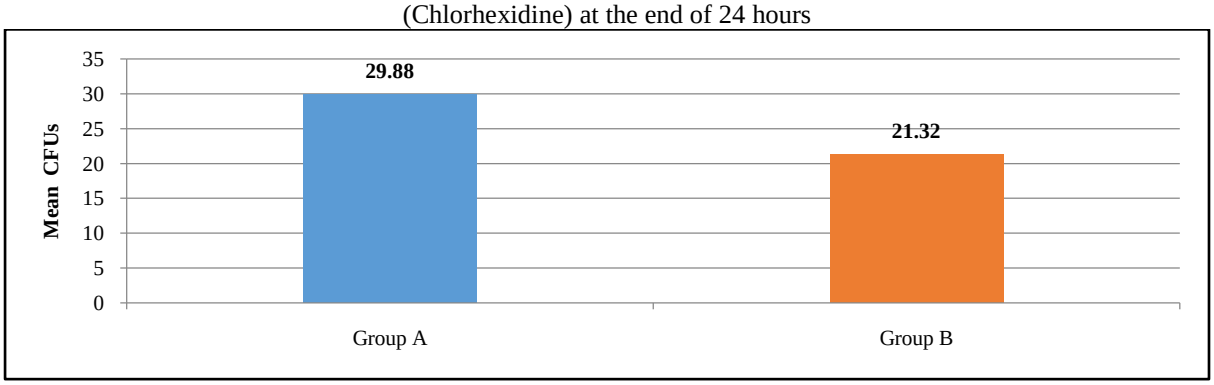


Table 6: Inter group comparison of mean CFUs at patient's chest area for Group A (Triphala) and Group B (Chlorhexidine) at the end of 24 hours

Groups	N	Total no. of CFUs at patient's chest area	Mean	Standard Deviation	Median	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Group A	25	1006	40.24	10.971	39	132	-3.507	0.000**
Group B	25	731	29.24	8.738	29			

Graph 6: Inter group comparison of mean CFUs at patient's chest area for Group A (Triphala) and Group B (Chlorhexidine) at the end of 24 hours

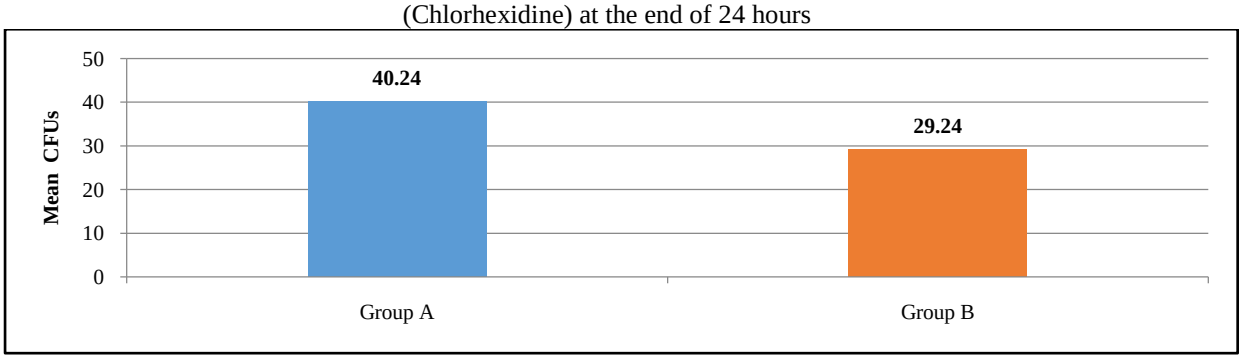


Table 7: Inter group comparison of mean CFUs at assistant's chest area for Group A (Triphala) and Group B (Chlorhexidine) at the end of 24 hours

Groups	N	Total no. of CFUs at assistant's chest area	Mean	Standard Deviation	Median	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Group A	25	574	22.96	9.190	21	147	-3.216	0.001**
Group B	25	376	15.04	6.195	13			

Graph 7: Inter group comparison of mean CFUs at assistant's chest area for Group A (Triphala) and Group B (Chlorhexidine) at the end of 24 hours

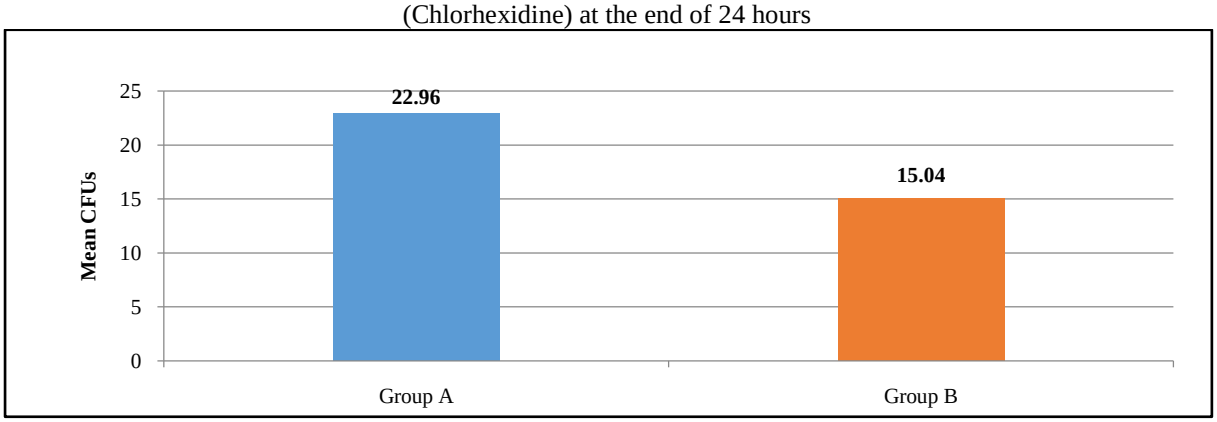


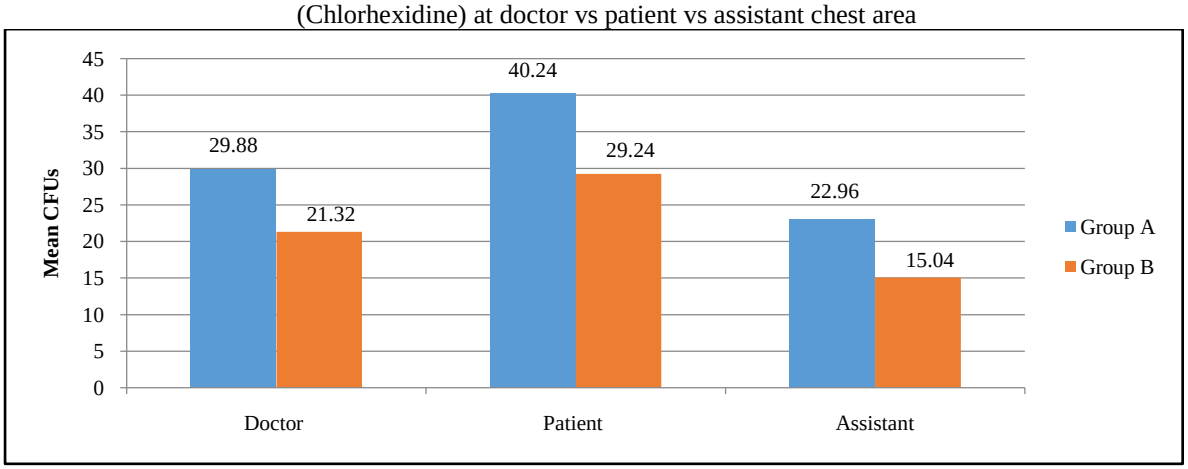
Table 8 (a): Intra group comparison of number of CFUs at doctor vs patient vs assistant chest area between Group A(Triphala) and Group B (Chlorhexidine)at the end of 24 hours

Chest area location	Groups	Total number of CFUs	Mean	Standard Deviation	Median	p value of Kruskal-Wallis Test
Doctor	**Group A**	747	29.88	9.567	30	
	Group B	533	21.32	6.902	20	
Patient	**Group A**	1006	40.24	10.971	39	0.000**
	Group B	731	29.24	8.738	29	
Assistant	**Group A**	574	22.96	9.190	21	
	Group B	376	15.04	6.195	13	

Table 8 (b): Pairwise comparison of CFUs at doctor vs patient , doctor vs assistant and patient vs assistant chest area between Group A (Triphala) and Group B (Chlorhexidine)

Pairwise comparison	Groups	Mann-Whitney U value	Z value	p value of Mann-Whitney U test
Doctor vs Patient	**Group A**	153.500	-3.088	0.002**
	Group B	150.500	-3.147	0.002**
Doctor vs Assistant	**Group A**	184.500	-2.487	0.013*
	Group B	151.000	-3.139	0.002**
Patient vs Assistant	**Group A**	68.000	-4.478	0.000**
	Group B	61.500	-4.876	0.000**

Graph 8: Intra group comparison of number of CFUs between Group A (Triphala) and Group B (Chlorhexidine) at doctor vs patient vs assistant chest area



IV. Discussion

Dentist, dental hygienist and oral health care workers practice in a highly contaminated environment which is the human mouth where they are exposed to variety of bacteria, viruses, fungi and protozoan from many sources.There are at least four potential sources of airborne contamination during dental treatment: dental instrumentation, saliva, respiratory sources, and the operative site.[7]One of the methods of reducing overall bacterial counts produced during dental procedures is preprocedural rinsing with a product containing an antimicrobial agents as a means of reducing aerosols generated by the ultrasonic scalers.

The analysis revealed that 10 ml 0.2% ofchlorhexidine preprocedural mouthwash when used as a mouthrinse for 1 minute, ten minutes before ultrasonic scaling showed a greater reduction of colony forming units (CFUs) at all the three locations i.e. doctor, patient and assistant chest area as compared to the 10 ml triphala preprocedural mouthwash used as a mouthrinse for 1 minute, ten minutes before ultrasonic scaling (the difference being statistically highly significant; $p<0.01$).

The results of the present study are in accordance with the various studies conductedby Gupta G, Mitra D, Ashok K, Gupta A, Soni S, Ahmed S, Arya A (2014)[1];Swaminathan Y, Thomas JT, Muralidharan NP (2014)[17]; Sethi G and Kumar K (2018)[18]and Yadav S, Kumar S, Srivastava P, Gupta K, Gupta J, Khan Y (2018)[19].

Rao R, Shenoy N, Shetty V (2015)[4]; Reddy S, Prasad MG, Kaul S (2012)[8]; Son WK, Shin SY, Kye SB, Yang SM (2009)[20]and Mohan M and Jagannathan N (2016)[21] reported that chlorhexidine can significantly reduce the viable microbial content of dental aerosols and protect the operator from the bacterial hazards. Studies reported by Logothetis (1995)[5] ; Sharma M, Srivastava A, Kumar V, Sharma M, Srivastava V, Srivastava S, Aamir M (2017)[22]and Tasneem S, Prasad S, Srinivas B, Kumar A, Yadav R (2017)[23], indicated that higher reduction in bacterial counts was achieved by the usage of chlorhexidine mouthwash as compared to other antimicrobial mouthwashes.

Malhotra R, Grover V, Kapoor A, Saxena D (2011)[24]andBhate D, Jain S, Kale R, Muglikar S (2015)[25]reported that chlorhexidine was considered to be more effective in reducing plaque as compared to herbal mouthwash. Haffajee AD, Yaskell T, Socransky SS (2008)[26] has found herbal to be a less potent antimicrobial agent than 0.12% CHX, but more effective than essential oil mouthrinse.The effectiveness of chlorhexidine depends not only on antimicrobial effects, but also oral retention, which prolongs antimicrobial action. CHX reduces salivary bacteria during scaling and root planing. Preprocedural rinsing with chlorhexidine has been shown to be of value in reducing salivary bacterial levels in the oral cavity, prior to dental treatment. Antimicrobial effects on total aerobes and anaerobes in saliva have been documented for as long as 5 hours after rinsing.

The results of this study are not in conjunction with studies by Maurya DK, Mittal N, Sharma KR, Nath G. (1997)[27] ; Kaim JM, Gultz J, Do L, Scherer W (1998)[28]and Tandon S, Gupta K, Rao S, Malagi KJ (2010)[29]asin their study triphala showed better results than chlorhexidine as far as plaque, gingival, and oral hygiene indices were concerned without any evidence of staining of teeth.The complex mechanisms of antibacterial activity of triphala include either inhibition of cell division or damage to the bacterial cell walls. Triphala inhibited the growth of all gram-positive and gram-negative bacteria. Oral rinsing with an extract of *Terminalis chebula* significantly reduce both total bacterial counts and streptococcal counts in saliva samples. The protective effect lasts for up to 3 hours after rinsing, demonstrating a potential role for *Terminalis chebula* in the reduction of salivary bacteria. Abraham S, Kumar M, Sehgal P, Nitish S, Jayakumar D (2005)[30]; Thomas B, Shetty SY, Vasudeva A, Shetty V (2011)[31] and Khalessi AM (2004)[32] concluded that triphala has significant antimicrobial activity and has strong inhibitory activity on MMPs involved in the extracellular matrix degradation during periodontitis.

On comparison of number of CFUs at doctor vs patient vs assistant chest area, the number of CFUs werehigher at the patient's chest area as compared to doctor's chestarea and assistant's chest area with higher mean CFUs for patient's chest area followed by doctor's chest area and least for assistant's chest area (the difference being statistically highly significant; $p<0.01$).

The highest CFU count at the patient's chest area is similar to the study conducted by Bentley CD, Burkhart NW, Crawford J (1994)[30]and Taksleem(1996)[72]as they observed that the larger salivary droplets generated during dental procedures settle rapidly from the air with heavy contamination on the patient's chest.

These observations reinforce the importance of using personal protective equipment like eye and face shields, head cap, mouth masks, gloves, gowns and validates the use of preprocedural mouthrinsing with an antimicrobial mouthwash as an additional barrier to minimize the risk of cross-contamination during ultrasonic scaling.

V. Conclusion

Although, as with all infection control procedures, it is impossible to completely eliminate the risk posed by dental aerosols, it is possible to minimize the risk by layering of protective procedures with relatively simple and inexpensive precautions. This study suggested that 10 ml of 0.2% chlorhexidine when used for ten minutes prior to ultrasonic scaling is more potent in reducing the aerosol contamination as compared to 10 ml of 0.6% triphala mouthwash. Also, the patient's chest area was more exposed to the microbial aerosols than that of the doctor's chest area, followed by that of the assistant's chest area.

Clinical transfer of this study result is that preprocedural rinsing should be made a regular practice in all dental set ups, along with high vacuum evacuation and other barrier techniques to prevent disease transmission through aerosols. The aerosol production cannot be totally eliminated during ultrasonic scaling, but the putative potential of these aerosols can be minimized by preprocedural rinsing. This reinforces the importance of using personal protective equipments by operator and assistants while caring out the procedures to prevent cross-infection**.**

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