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ORIGINAL ARTICLE

CHLORHEXIDINE AND *MORINDA CITRIFOLIA* MOUTHWASH AS A PREPROCEDURAL MOUTHRINSE DURING ULTRASONIC SCALING – A RANDOMISED CLINICAL TRIAL

Clorhexidina y *Morinda citrifolia* como enjuague bucal profiláctico previo al raspado ultrasónico: un ensayo clínico aleatorizado

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ABSTRACT

Background: Aerosols released during ultrasonic scaling can cause cross-contaminations. Infections like hepatitis and SARS-CoV-2 can spread through these aerosols. Pre-procedural rinse is a cost-effective method to reduce aerosol contami-nation produced during ultrasonic scaling.

Materials and Methods: This was a double-blinded randomised trial on 105 subjects diagnosed with chronic generalised periodontitis which was randomly allocated into three groups: Group I received 0.12% chlorhexidine (CHX), Group II received 5% Morinda citrifolia (noni) mouthrinse, Group III received distilled water. Participants were advised to mouthrinse for 60 seconds just before the start of ultrasonic scaling. The aerosols released during ultrasonic scaling were gathered on blood agar plates placed at three locations. Colony forming units (CFUs) were counted after 48 hours of incubation.

Results: The highest CFU values were observed in the distilled water group and the lowest in chlorhexidine group (p<0.05). Patient chest area has the highest number of CFU than doctors' and assistant chest area (p<0.05).

Conclusions: Preprocedural rinsing with noni mouthwash can be used as an effective method of reducing aerosol contamination during ultrasonic scaling.

Keywords: *Aerosols; Chlorhexidine; Morinda citrifolia; mouthwashes; Dental prophylaxis; Dental scaling.*

RESUMEN

Antecedentes: Los aerosoles liberados durante el raspado ultrasónico pueden provocar contaminaciones cruzadas. Infecciones como la hepatitis y el SARS-CoV-2 pueden propagarse a través de estos aerosoles. El enjuague previo al procedimiento es un método rentable para reducir la contaminación por aerosoles producida durante el raspado ultrasónico.

Materiales y Métodos: Este fue un ensayo aleatorio doble ciego en 105 sujetos diagnosticados con periodontitis crónica generalizada que fueron asignados aleatoriamente en tres grupos: Grupo I: recibió clorhexidina (CHX) al 0,12%, Grupo II: recibió enjuague bucal de Morinda citrifolia (noni) al 5% ,Grupo III: recibió agua destilada. Se recomendó a los participantes que se enjuagaran la boca durante 60 segundos justo antes del inicio del raspado ultrasónico. Los aerosoles liberados durante el escalado ultrasónico se recolectaron en placas de agar sangre colocadas en tres lugares. Las unidades formadoras de colonias (UFC) se contaron después de 48 horas de incubación.

Resultados: Se observaron valores de UFC más altos en el grupo de agua destilada y más bajos en el grupo de clorhexidina (p<0,05). El área del tórax del paciente tuvo el mayor número de UFC comparando con el área del tórax de los odontologos y asistentes (p<0,05).

Conclusión: El enjuague previo al procedimiento con enjuague bucal de noni se puede utilizar como un método eficaz para reducir la contaminación por aerosoles durante el raspado ultrasónico.

Palabras Clave: *Aerosoles; Clorhexidina; Morinda citrifolia; Antisépticos bucales; Profilaxis dental; Raspado dental.*

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INTRODUCTION

Aerosols are generated during dental procedures like ultrasonic scaling, use of air-rotors during cavity preparation or root canal treatment. These aerosols can be sustained in the environment for a long duration and since its very tiny particle size of 1-5µm, invisible to the naked eye.[1] Identification of particles and to prevent them from cross contamination remains a challenge. Various guidelines have been published to reduce aerosol load in a dental setting.

Rubber dam placement, high velocity air evacuation, proper patient positioning are few commonly used methods.[2] Preprocedural rinsing is a cost-effective method of reducing aerosol contamination, it can be used alone or in combination with other methods to achieve aerosol reduction,[3] 0.12% chlorhexidine (CHX), 1% povidone iodine, herbal mouthwashes has been used as preprocedural rinse in reducing in aerosol contamination.[4]

Infection control protocols includes proper ventilation of the dental clinic, use of sterilised instruments, proper waste management protocols, use of gloves, double masks, protective eyewear.[5] As Villani *et al.*,[6] stated, dentistry is one of the most exposed professions to respiratory diseases, as such proper infection control guidelines need to be implemented to prevent aerosol prevention. Most of the dental procedures are related to high or low aerosol production and efforts should be made to reduce the cross contamination and risk of infectious diseases.

Recently, boric acid mouthwash was used as a preprocedural rinse and was found effective in reducing aerosols during ultrasonic scaling.[7]

Herbal mouthwash is a safe substitute of available mouthrinse as it has fewer side effects like minimal to no staining,unpleasant taste, alteration to salivary gland secretion. Many herbal mouthwashes like aloe vera, turmeric, triphala, neem, tree tea, Punica granatum has been used in dentistry as an effective antiplaque agent.[8]

Morinda citrifolia L. (MC) has been used as juice, it has anti-inflammatory and antioxidant properties.[9] Murray et al. used MC juice as an intracanal irrigant and found its efficacy similar to sodium hypochlorite.[10]

Another study by Glang *et al.*,[11] evaluated effects of MC juice as anti-gingivitis agent and found significant reduction in gingival inflammation. Few very studies have evaluated effect of MC as mouthwash and no preprocedural mouthrinse has been proven superior to chlorhexidine and so the present study compared efficacy of MC mouthwash with CHX as preprocedural rinse in reducing ultrasonic scaling contaminating aerosols.

The null hypothesis of the study was that there lies no difference in efficacy of MC mouthwash with CHX as preprocedural rinse in reducing aerosols produced during ultrasonic scaling.

MATERIALS AND METHODS

This study was conducted at the Department of Periodontology, Haldia Institute of Dental Sciences and Research, Haldia,West Bengal from January 2022 to February 2022.

The study was approved by the Institutional Ethics Committee. It was registered at Clinical Trial Registry India -CTRI/2022/02/040004.

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Written informed consent was taken before starting the study. Patients aged between 35-55 years both male and female were considered.

Morinda citrifolia mouthwash – Preparatory phase

The *Morinda citrifolia* plant was identified and authenticated by the Dept. of Pharmacognosy, Haldia Institute of Pharmacy, Haldia, West Bengal, India. *Morinda citrifolia* fruit was collected from a local nursery in Kolkata. The fruit was cleaned and dried at temperature 400 C at Haldia Institute of Pharmacy, Haldia, West Bengal. The dried form was stored until further use. This dried fruit was mashed with ethanol 96% and a thick texture was obtained by rotary evaporator.

A 5% *Morinda citrifolia* mouthwash was prepared by combining 10 g of MC fruit extract with 200 mg sodium benzoate, 600 mg sodium saccharin, 100 mg acidum benzoate, 200 mg Tween 80, 2 ml oleum menthae, 2 mg blue dye and 200 ml distilled water, in accordance with the method described by Aldi *et al.*[12]

Inclusion criteria were:

i) periodontitis diagnosed with stage II, grade A;
ii) mean plaque index(PI)13 score of 2.0 - 3.0.

Exclusion criteria were:

i) Participants with history of hypersensitivity to any ingredients of chlorhexidine gluconate and;
ii) smokers;
iii) pregnancy/lactating mother;
iv) patient on antimicrobial/anti-inflammatory/ immunomodulatory drugs in last six months were excluded from the study.

Sample size was determined based on the pilot study on a similar population. The power of the study was at 90% and at 5% significance level, 30 subjects per group was required. Taking an attrition rate of 10% into consideration, a total of 35 subjects in each group were selected.

Randomisation and allocation

A computer generated random method was used and 1:1:1 allocation ratio was used. Block randomisation was done. Sequentially numbered opaque containers were used for allocation. Clinicians not involved in any clinical procedure and measurement were responsible for randomisation and allocation.

Blinding

This was a double-blind study both the clinician and patients were blinded to the mouthrinse used before scaling.

Intervention

A closed dental operating room was used for ultrasonic scaling to avoid any source of cross contamination. The room was disinfectant with fumigant after each session.

1) Group I, 35 subjects received 0.2% chlorhexidine mouthwash (10 ml) (Rexidine, Warren Group, India) as preprocedural mouthrinse
2) Group II, 35 subjects received 5% *Morinda citrifolia* mouthwash (10 ml) as preprocedural mouthrinse
3) Group III, 35 subjects received distilled water (DW) as preprocedural mouthrinse

Agar plate positions

Blood agar plates were placed at three positions from patients' mouth as a reference point.

a) Patient's chest area.
b) Operator's chest area.
c) Dental assistant chest area.

Each patient was instructed to rinse with 10ml of CHX/*Morinda citrifolia*/ distilled water as mouth rinse for 60 seconds. After 10 minutes, ultrasonic scaling with a high-volume saliva ejector was placed and randomly selected quadrant scaling was done for 20 minutes. Agar plates at three positions were left to collect the remaining aerosols suspended in air.

Microbiological analysis

Agar plates were incubated at 37°C for 48 hours and the total number of colony forming units (CFUs) were counted at the microbiology department of Institute of Medical Sciences and Research, Haldia.

Statistical Analysis

Descriptive analysis was expressed in mean and standard deviation (Table 1). CFU's comparison was done using analysis of variance (ANOVA). Statistical analysis was done using SPSS software (SPSSforWindows, Version22.0, SPSSInc., Chicago,IL, USA).

Independent *t*-test was used to evaluate mean differences between the groups; $p<0.05$ was considered statistically significant.

RESULTS

A total of 105 subjects were randomised into three groups: 35 subjects in each group, no dropouts occurred during the study (Figure 1).

No significant difference ($p>0.05$) was observed between the three groups in relation to age, gender and plaque index (Table 1). No adverse side effects were noted post rinsing with any mouthwash in any subjects. Group I showed statistically significant reduction in CFUs count ($p<0.05$) compared to group II and group III (Figure 2).

Morinda citrifolia mouthrinse showed reduction in CFU count when compared to distilled water ($p<0.05$) (Table 2). The highest CFU was found in patients' chest area followed by operators' chest area and assistants' chest area (Table 3).

DISCUSSION

Dental procedures require close contact with patients' mouths and this brings the operator, assistant and other patients at high risk of cross infections. The droplets generated can get contaminated with the patient's saliva and risk of infectious disease transmission is high. Efforts should be made to reduce air-borne disease transmission in the dental set up.

After the outbreak of COVID-19 various modified healthcare suits and sprayhoods are being studied which can be used in day-to-day dental practice also.[14] Personal protective devices use should be encouraged by health care workers and initiatives should be taken towards awareness and prevention of aerosol contamination.

A recent study by Haffner *et al.*,[15] found that the three-dimensional splatter produced is of high velocities (1–2 m/s) seen below the intersection point between the front teeth and the scaler that propagated 10mm –15mm away from the location of the scaler tip.

High velocity isolated droplets propagate away from the bulk of the splatter. These findings suggest use of combined methods of aerosol reduction can be advantageous in limiting the aerosol contamination during scaling.

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Figure 1. Consort flowchart of the study.

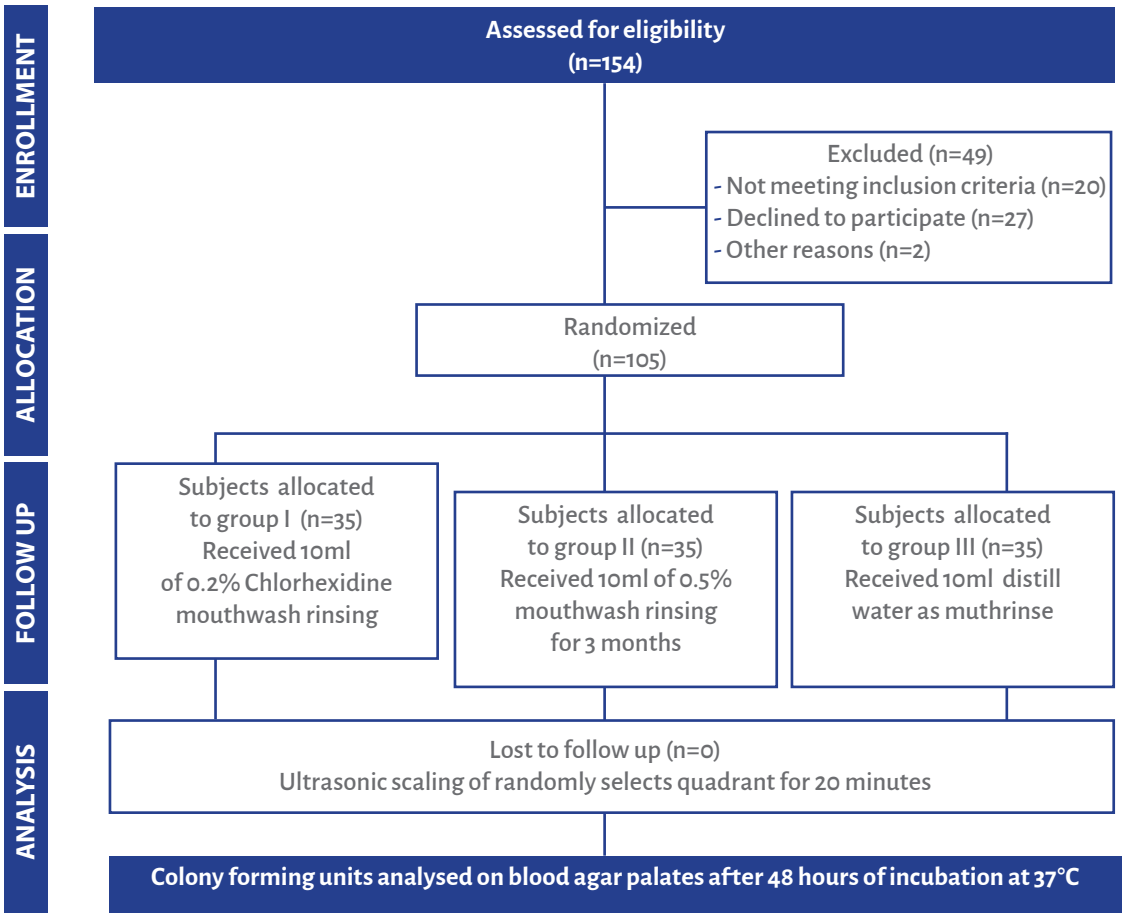
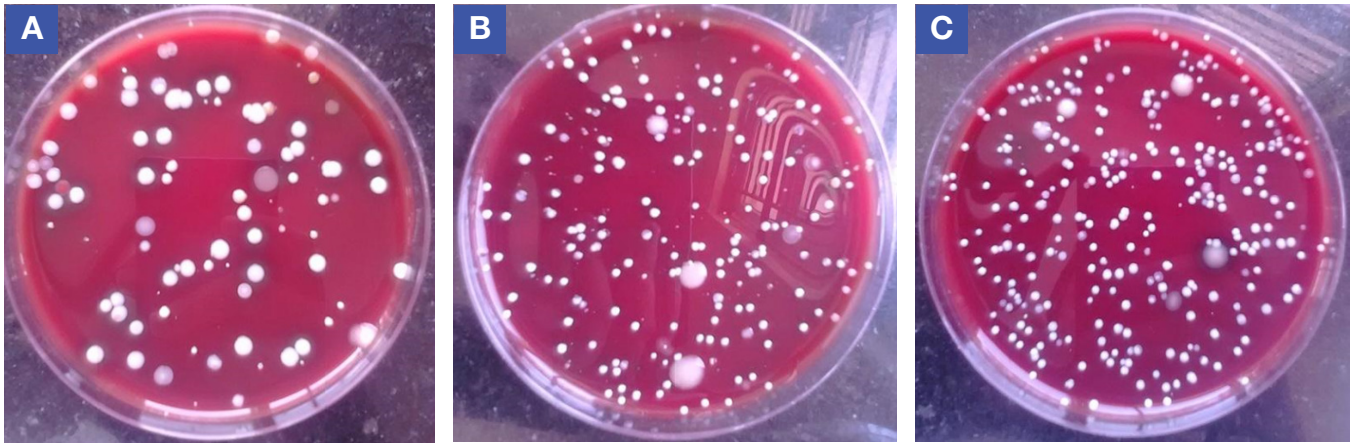


Figure 2. Blood agar plates showing colonies after incubation, after using preprocedural rinsing with different fluids.



A.Chlorhexidine mouthrinse. **B.** Morinda citrifolia mouthrinse. **C.** Distilled water.

Table 1. Baseline demographic and clinical characteristics of each group.

Variable	Group 1 (0.2% CHX)	Group 2 (5%MC)	Group 3 (Distilled water)	*p*-value
Age	39.37 ±3.18	41.23±4.12	38.13±5.23	NS
Male/female	16/19	15/20	17/22	NS
No. of teeth	26.22 ±1.12	27.11 ±1.32	26.14±1.12	NS
Plaque Index	2.13± 0.03	2.12± 0.04	2.17±0.05	NS

MC. Morinda citrifolia L. **NS.** Non-Significant. **CHX.** Chlorhexidine.

Table 2. Colony forming units (CFU) counts in relation to treatment and location.

Location of Agar Plate	Number of CFUs (Mean± Standard Deviation)		
	Group I	Group II	Group III
Doctor's chest	33.5±3.60	48.4 ±2.10	82.15±4.15
Assistant's chest	23.11 ±1.05	35.12±2.13	55.16±2.15
Patient' chest	92.12±3.19	153.32±3.12	232.32 ±5.32

Table 3. Comparison of colony forming units (CFU) counts at all three locations.

Location	Mouthrinse	Mean±S.D	F-Value	*p*-value
Doctor's chest	Chlorhexidinet	33.5±3.60	1,885.905	<0.001
	Morinda citrifolia L	48.4 ±2.10		
	Distilled Water	82.15±4.15		
Patient's chest	Chlorhexidinet	92.12±3.19	10,759.432	<0.001
	Morinda citrifolia L	153.32±3.12		
	Distilled Water	232.32 ±5.32		
Assistant's chest	Chlorhexidinet	23.11 ±1.05	2,682.571	<0.001
	Morinda citrifolia L	35.12±2.13		
	Distilled Water	55.16±2.15		

SD. Standard Deviation.

In the present study preprocedural mouthrinse was used along with high suction as a method to reduce aerosol contamination. We hypothesised that *Morinda citrifolia* mouthwash is effective in reducing aerosol contamination when used as preprocedural rinse. This hypothesis was based on previous studies on Morinda citrifolia as anti-gingivitis agent suggesting its antimicrobial property.[16]

A recent study on use of *Morinda citrifolia* mouthwash in gingival inflammation showed significant re-duction in gingival index and plaque index at three months follow up.[17] In the present study we evaluated potential of *Morinda citrifolia* mouthwash as preprocedural mouthrinse and found significant reduction in CFU counts compared to control group (distilled water). This can be attributed

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to the antimicrobial action of *Morinda citrifolia* against the aerosol released during scaling. The aerosol released during scaling consists of microbes like fungi, bacteria and virus from oral cavity, saliva, blood, gingiva and dental plaque.[18]

Water from dental units might also contribute towards pathogenic microorganisms in aerosols. The aerosol when inhaled can be infectious and it can remain suspended for hours in dental office, which can cause delayed infections.[19]

When we compare 0.2% chlorhexidine mouthwash efficacy against 5% *Morinda citrifolia* mouthwash as preprocedural rinse, we found CHX mouthwash as superior preprocedural rinse as CFU count were significantly less in Group I compare to Group II ($p<0.05$). This may be due to the potential bacteriostatic and bactericidal action of CHX.[20]

Location-wise, the patient chest area had the highest CFUs suggesting that the aerosols are suspended more closer in areas near the oral cavity. This was followed by operators' chest area as the operator works in close association with the patient's mouth to gain accessibility to the working field and the lowest CFUs were seen in the assistant chest area. These locations were decided based on the study results Mirhoseini *et al.*,[21] stating mean concentration of airborne bacteria lies between 52–1030 and 8–844 CFU/m^3 at the distances of 0.5 and 2 m, respectively.

Another study by Zemouri *et al.*,[22] on spatial distribution of aerosols lies within 80 cm around the head of the patient and so in the present study all the agar plates at the three locations were kept as close to 1 m distance from patient's mouth as a reference point.

Similar results were seen by Gupta *et al.*,[23] in their study on herbal mouthrinse and 0.2% chlorhexidine gluconate, total number of colony forming units were lower in CHX group compared to herbal mouthwash. Paul *et al.*,[24] used aloe vera mouthwash as preprocedural rinse and found it effective in reducing CFUs count.

They also evaluated herbal mouth-rinse suggesting its safe properties compared to CHX. Retamol *et al.*,[25] studied mouthwash containing cetylpyridinium chloride, zinc lactate and sodium fluoride, which generated 70% less aerosol as compared to water and no rinse group.

This study highlighted that though CHX is preferred during different dental procedures, in cases of hypersensitivity or allergic reactions to CHX, substitutes with potential antimicrobial action and minimal side effects are needed to be studied. A systematic review by Nagraj *et al.*,[26] on preprocedural rinse suggested that regular use can effectively reduce the aerosol contamination in dental clinics.

A meta-analysis on use of herbal products in dentistry suggested that herbal products have anti-inflammatory actions and can act as ant gingivitis agent, this potential of herbal agents needs to be explored further by multicentre trials evaluating its antimicrobial actions.[27] Kumar *et al.*,[28] suggested method to mitigate infection spread from aerosol and they highlighted the use of preprocedural rinse as an effective method in reducing aerosol contamination. In the present study

we found preprocedural rinsing reducing CFU counts as compared to control group. Verbeek *et al.*,[29] suggested use of personal protective equipment to prevent transmission of infectious diseases.

Alexendra *et al.*,[30] did a quantitative assessment of aerosol produced during ultrasonic scaling and found airborne particles 0-1.2 m and 1.2-2.4 m from patients were produced during *in vivo* ultrasonic scaling with a saliva ejector This finding highlights the need of additional source along with saliva ejector in reducing aerosol contamination. Combination methods should be used whenever possible in combating aerosol contamination. Several challenges like economic restraints, dental clinic area, ventilation, total number of patient inflow, duration of procedure, patients' general health status and immunity comes into play when we sum-up the possible factors associated with aerosol contamination.[31]

Limitation

The present study evaluated only aerobic colony forming units because of lack of infrastructure to grow anaerobic culture. Future studies should focus on viruses, anaerobic microorganisms through selective sampling and culturing methods. Also, sampling time variations were not accounted for so the results cannot be generalised. As aerosols are heterogeneous, the distance, location should be also considered while interpreting the results.

CONCLUSION

It can be concluded from the present study that CHX, as a gold-standard anti-plaque agent, had the highest degree of antimicrobial properties.

However, *Morinda citrifolia*, having minimal side effects compared to CHX, could be used as an alternative to CHX as preprocedural mouthrinse.

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CONFLICT OF INTERESTS

Authors declare that they have no conflicts of interest.

ETHICS APPROVAL

The study was approved by the Institutional Ethics Committee. It was registered at Clinical Trial Registry India -CTRI/2022/02/040004.

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None.

AUTHORS' CONTRIBUTIONS

Swet Nisha: Concepts, Design, Literature search, Clinical studies, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manus-cript preparation, Manuscript editing, Manuscript review.
Debanjan Das: Definition of intellectual content; Concepts, Design, Literature search, Data acquisition, Manuscript preparation, Manuscript review.
Pratibha Shashikumar: Design, Literature search, Data analysis, Manuscript editing, Manuscript review.

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