

TRIAL PROTOCOL AND PARTICIPANT INFORMED CONSENT FORM

Official Title of the Study

Feasibility and Preliminary Clinical Outcomes of Alum, Triphala, and Chlorhexidine Mouth Rinses During Clear Aligner Therapy: A Pilot Randomized Controlled Trial

ClinicalTrials.gov Identifier (NCT Number): Not available - study was not prospectively registered

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Faridabad, India

TRIAL PROTOCOL

Title:

Feasibility and Preliminary Clinical Outcomes of Alum, Triphala, and Chlorhexidine Mouth Rinses During Clear Aligner Therapy: A Pilot Randomized Controlled Trial

Study Design:

Pilot, parallel-arm, randomized controlled clinical trial

Trial Setting:

Department of Orthodontics and Dentofacial Orthopaedics, Manav Rachna Dental College, Manav Rachna International Institute of Research and Studies (MRIIRS), Faridabad, India

Administrative Information**Principal Investigator:**

Dhruv Ahuja

Co-Investigators:

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Ethics Approval:

Institutional Ethics Committee, Manav Rachna Dental College
Ref. No. MRIIRS/MRDC/SDS/IEC/2024/72

Funding:

No external funding

Trial Registration:

Not prospectively registered in a public clinical trial registry (pilot investigator-initiated study)

1. Background and Rationale

Clear aligner therapy has emerged as an increasingly popular orthodontic treatment modality due to its esthetic advantages, removability, and improved patient comfort compared to fixed appliances. However, despite these advantages, aligner therapy does not fully eliminate the risk of plaque accumulation and gingival inflammation, particularly due to the presence of bonded composite attachments that act as plaque-retentive niches.

Orthodontic patients are at increased risk of plaque-induced gingivitis due to changes in oral hygiene efficiency and microbial biofilm retention. Mechanical plaque control alone may be insufficient in such cases, and chemical adjuncts such as mouth rinses are frequently recommended.

Chlorhexidine gluconate is considered the gold standard antiplaque agent due to its substantivity and broad-spectrum antimicrobial activity. However, its long-term use is limited by adverse effects such as tooth staining, altered taste perception, and mucosal irritation.

Herbal alternatives such as Triphala, a traditional Ayurvedic formulation composed of *Terminalia chebula*, *Terminalia bellirica*, and *Emblica officinalis*, have demonstrated antimicrobial, antioxidant, and anti-inflammatory properties in previous studies. Alum (potassium aluminum sulfate) has also been reported to possess astringent and antimicrobial effects that may reduce plaque accumulation.

Despite existing evidence, there is a lack of clinical trials evaluating these agents specifically in patients undergoing clear aligner therapy, where biofilm dynamics differ from conventional orthodontic conditions.

2. Objectives

Primary Objective:

To evaluate the effect of alum, Triphala-based herbal, and 0.2% chlorhexidine mouth rinses on gingival inflammation (Gingival Index) in patients undergoing clear aligner therapy.

Secondary Objectives:

- To compare changes in Plaque Index (PI) among the three groups
- To assess changes in salivary *Streptococcus mutans* counts
- To evaluate feasibility outcomes including recruitment, retention, and compliance
- To estimate variability and preliminary effect sizes for future definitive trials

3. Hypothesis

Null Hypothesis:

There is no significant difference among alum, Triphala, and chlorhexidine mouth rinses in reducing plaque accumulation, gingival inflammation, or salivary *S. mutans* levels.

Alternative Hypothesis:

At least one mouth rinse produces a significant difference in clinical or microbiological outcomes.

4. Trial Design

This study was designed as a **pilot, randomized, parallel-group controlled clinical trial** with three intervention arms in a 1:1:1 allocation ratio.

- Arm 1: Alum mouth rinse (0.02 M potassium aluminum sulfate)
- Arm 2: Triphala-based herbal mouth rinse
- Arm 3: 0.2% chlorhexidine gluconate mouth rinse

Participants were followed for a total duration of **30 days**, with assessments at baseline (T0), 15 days (T1), and 30 days (T2).

5. Participants

5.1 Study Population

Participants were recruited from patients undergoing active clear aligner orthodontic treatment at the Department of Orthodontics and Dentofacial Orthopaedics, Manav Rachna Dental College, MRIIRS, Faridabad, India.

All participants had been using clear aligners for at least 4 weeks prior to enrolment and had bonded composite attachments placed on multiple teeth.

5.2 Inclusion Criteria

Participants were included if they met the following criteria:

- Age 18–35 years
- Undergoing active clear aligner therapy
- Wearing aligners for ≥ 20 –22 hours/day
- Presence of mild to moderate plaque-induced gingivitis (Löe and Silness Gingival Index 1–2)
- Presence of at least 20 natural teeth

- Willingness to comply with study protocol and follow instructions
- Signed informed consent

5.3 Exclusion Criteria

Participants were excluded if they had:

- Antibiotic use within the past 3 months
- Use of antimicrobial mouth rinses within the past 1 month
- History of periodontal pockets >3 mm
- Systemic diseases affecting periodontal health
- Smoking or tobacco use
- Pregnancy or lactation
- Known allergy to any study mouth rinse components
- Ongoing systemic medication affecting salivary flow or gingival status

6. Recruitment and Enrollment

Participants were recruited consecutively from the orthodontic outpatient clinic. Eligibility was assessed through clinical examination and medical history review.

All eligible participants were provided with detailed information regarding the study purpose, procedures, risks, and benefits. Written informed consent was obtained prior to enrolment.

7. Sample Size

As this was a **pilot feasibility trial**, formal power calculation for hypothesis testing was not performed.

A pragmatic sample size of **30 participants (10 per group)** was selected based on methodological recommendations for pilot randomized controlled trials. This sample size was considered adequate to:

- Assess feasibility parameters (recruitment and retention rates)
- Estimate variability in clinical outcomes
- Generate preliminary effect size estimates for future definitive trials

No interim analysis or stopping rules were applied due to the short study duration.

8. Randomization and Allocation Concealment

Participants were randomly allocated into three groups in a **1:1:1 ratio** using computer-generated block randomization (block size = 3).

An independent investigator generated the random allocation sequence.

Allocation concealment:

- Sequentially numbered opaque sealed envelopes (SNOSE) were used
- Envelopes were prepared by an independent investigator not involved in recruitment or outcome assessment
- Allocation was revealed only after participant enrolment

9. Blinding

Due to the distinct taste and sensory characteristics of the mouth rinses, **participant blinding was not feasible**.

However:

- Outcome assessors were blinded to group allocation
- Laboratory personnel were blinded during microbiological analysis
- Statistical analysis was performed blinded to group assignment

10. Interventions

Participants were allocated to one of three intervention groups:

Group 1: Alum Mouth Rinse

- Composition: 0.02 M potassium aluminum sulfate solution
- Source: HiMedia, India
- Preparation: Prepared under standardized laboratory conditions
- Dose: 10 mL per rinse
- Frequency: Twice daily (morning and evening after brushing)
- Duration: 30 days
- Rinsing instructions: Swish for 60 seconds, then expectorate

Group 2: Triphala-based Herbal Mouth Rinse

- Commercial preparation: Planetary Herbals, USA
- Composition: Triphala herbal extract (*Terminalia chebula*, *Terminalia bellirica*, *Embllica officinalis*)
- Dose: 10 mL per rinse
- Frequency: Twice daily
- Duration: 30 days
- Rinsing instructions: Swish for 60 seconds, then expectorate

Note: The exact phytochemical concentration and vehicle composition were not fully standardized due to commercial formulation variability.

Group 3: Chlorhexidine Mouth Rinse

- Composition: 0.2% chlorhexidine gluconate solution
- Manufacturer: Basic Pharma Life Science, India
- Dose: 10 mL per rinse
- Frequency: Twice daily
- Duration: 30 days
- Rinsing instructions: Swish for 60 seconds, then expectorate

General Instructions for All Groups

Participants were instructed:

- To avoid eating or drinking for at least 30 minutes after rinsing
- To continue routine oral hygiene practices using fluoridated toothpaste and their usual toothbrush
- Not to use any additional mouth rinses or antimicrobial agents during the study period
- To maintain aligner wear for 20–22 hours/day

11. Outcome Measures

11.1 Primary Outcome

- Change in Gingival Index (Löe and Silness) from baseline to 30 days

11.2 Secondary Outcomes

- Change in Plaque Index (Silness and Løe)
- Change in salivary *Streptococcus mutans* counts ($\log_{10}$ CFU/mL)
- Percentage reduction in PI and GI from baseline to 30 days
- Compliance rate
- Feasibility outcomes (recruitment, retention, adherence)

12. Clinical Assessment

Clinical evaluations were performed at:

- T0: Baseline
- T1: 15 days
- T2: 30 days

Indices used:

Plaque Index (Silness and Løe)

Assessed plaque thickness at the gingival margin on a 0–3 scale.

Gingival Index (Løe and Silness)

Assessed gingival inflammation on Ramfjord index teeth (16, 21, 24, 36, 41, 44).

All examinations were performed using a William's periodontal probe under standardized lighting by a calibrated examiner blinded to group allocation.

13. Microbiological Assessment

Saliva Collection

- Paraffin-stimulated saliva samples collected
- Morning collection, at least 2 hours after food intake
- Volume: approximately 2 mL
- Stored in sterile containers
- Transported within 1 hour under cooled conditions

Culture Method

- Medium: Mitis Salivarius Bacitracin (MSB) agar
- Incubation: 37°C, 5% CO₂, 48 hours
- Outcome: Colony-forming units (CFU/mL)
- Data transformation: $\log_{10}$ CFU/mL prior to statistical analysis

Note on Method Limitations

MSB agar provides selective isolation of mutans streptococci but does not offer species-level molecular specificity and may allow growth of closely related oral streptococci.

14. Compliance Monitoring

Compliance was assessed using:

- Daily self-reported rinse logs
- Measurement of returned bottle weight at follow-up visits

Compliance rate was calculated as percentage adherence to prescribed usage.

15. Statistical Analysis Plan

All statistical analyses were performed using SPSS version 29 (IBM Corp., Armonk, NY, USA).

Data handling:

- Continuous variables summarized as mean $\pm$ SD and median (IQR) where appropriate
- Normality assessed using Shapiro–Wilk test

Inferential analysis:

As data were non-normally distributed, non-parametric tests were applied:

- **Within-group comparisons over time:** Friedman test
- **Post hoc analysis:** Dunn’s test with Bonferroni correction
- **Between-group comparisons at each time point:** Kruskal–Wallis test
- **Post hoc between-group comparisons:** Dunn’s test with Bonferroni adjustment (where applicable)

Additional analyses:

- Percentage reduction from baseline to 30 days was calculated for PI and GI
- Statistical significance set at $p < 0.05$ (two-tailed)

16. Ethical Considerations

The study was approved by the Institutional Ethics Committee of Manav Rachna Dental College, MRIIRS, Faridabad, India (Ref. No. MRIIRS/MRDC/SDS/IEC/2024/72).

All procedures were conducted in accordance with:

- The Declaration of Helsinki
- Institutional ethical guidelines
- Good Clinical Practice principles

Written informed consent was obtained from all participants prior to enrolment.

Participants were informed about:

- Study objectives
- Potential benefits and risks
- Right to withdraw at any time without penalty

No adverse events were reported during the study period.

17. Data Management

All participant data were anonymized using unique study identification codes.

Data handling procedures included:

- Secure storage of paper records in locked cabinets
- Password-protected electronic databases
- Restricted access to authorized investigators only

Data integrity was maintained throughout the study, and no identifiable personal information was used in analysis or reporting.

18. Dissemination Plan

Study findings will be disseminated through:

- Publication in peer-reviewed scientific journals
- Presentation at national and international conferences
- Academic and clinical orthodontic forums

No participant-level identifiable data will be shared.

19. Protocol Amendments

No major protocol amendments were made after ethics approval. Any minor procedural clarifications were documented and maintained within study records.

20. Declaration of Trial Registration Status

This investigator-initiated pilot randomized clinical trial received Institutional Ethics Committee approval prior to participant recruitment; however, it was not prospectively registered in a public clinical trial registry.

This limitation is acknowledged in the manuscript and considered in the interpretation of findings.

PARTICIPANT INFORMED CONSENT FORM (PICF)

Protocol / Study number : _____

Participant identification number for this trial: _____

Title: of project: Feasibility and Preliminary Clinical Outcomes of Alum, Triphala, and Chlorhexidine Mouth Rinses During Clear Aligner Therapy: A Pilot Randomized Controlled Trial

Name of Principal Investigator: ____Dr Dhruv Ahuja____Tel.No(s).____8722122484

The contents of the information sheet dated that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

The nature and purpose of the study and its potential risks / benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from _____. I give permission for these individuals to have access to my records.

I agree to take part in the above study.

----- Date: (Signatures / Left Thumb Impression) Place:

Name of the Participant: _____

Son / Daughter / Spouse of: _____

Complete postal address: _____

This is to certify that the above consent has been obtained in my presence.



Signatures of the Principal Investigator

Date:

Place:

1) Witness – 1

2) Witness – 2

Signatures

Signatures

Name:

Name:

Address:

Address

