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*Review Article*

# Comparison Of Streptococcus Mutans Count in Orthodontic Patients Using Herbal Vs. Calcium Sucrose Phosphate Toothpaste

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**Abstract**
Orthodontic therapy utilizing fixed appliances such as stainless-steel brackets is inherently associated with biofilm accumulation, creating a conducive microenvironment for acidogenic and aciduric microorganisms most notably *Streptococcus mutans*, the primary etiological agent of dental caries. This longitudinal, interventional clinical trial was designed to evaluate and compare the anti-microbial efficacy of a neem-based phytotherapeutic dentifrice versus a calcium sucrose phosphate-enriched remineralizing toothpaste in orthodontic patients over a six-month period. Thirty participants undergoing fixed orthodontic treatment were randomly stratified into two groups (n=15 each). Group A received a standardized neem-based herbal toothpaste, while Group B utilized a formulation containing calcium sucrose phosphate. Plaque samples were aseptically collected at the 3rd and 6th months, followed by microbial quantification using Mutans-Sanguis agar and colony-forming unit (CFU) enumeration under optical microscopy. Statistical analysis revealed that both dentifrices produced a significant intragroup reduction in *S. mutans* count ($p < 0.05$). However, Group B exhibited a greater decline (56.94%) compared to Group A (42.74%), with intergroup comparisons at both time points showing statistically significant differences ($p < 0.05$). The enhanced efficacy of calcium sucrose phosphate may be attributed to its dual action disrupting bacterial colonization and promoting enamel remineralization by supplying bioavailable calcium and phosphate ions. Meanwhile, neem's antimicrobial bioactives like azadirachtin and nimbidin demonstrated moderate but sustained bacteriostatic effects. This study underscores the clinical significance of selecting dentifrices with both antimicrobial and remineralizing properties during fixed orthodontic therapy. The incorporation of evidence-based oral hygiene adjuncts can mitigate the microbial dysbiosis induced by orthodontic appliances and reduce the risk of enamel demineralization, ultimately enhancing long-term oral health outcomes.

**Keywords:** *Streptococcus mutans*, Calcium sucrose phosphate, Neem toothpaste, Fixed orthodontic appliances, Remineralization

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## 1. Introduction

Oral health is a crucial aspect of general well-being, influencing not only the physical ability to eat and speak but also social interaction and overall quality of life[1]. Maintaining proper oral hygiene becomes even more essential in individuals undergoing orthodontic treatment, particularly those fitted with fixed appliances such as stainless steel brackets. These appliances create retention sites for plaque accumulation, making it challenging to maintain oral cleanliness[2]. One of the most problematic bacteria associated with dental caries is *Streptococcus mutans*, which thrives in plaque and contributes to enamel demineralization through lactic acid production. In orthodontic patients, the count of *S. mutans* tends to be significantly higher due to the difficulty in plaque removal around brackets and wires[3]. This creates a heightened risk for the development of white spot lesions, gingivitis, and other periodontal complications, making the prevention of bacterial proliferation a primary focus during orthodontic treatment[4]. Orthodontics, the branch of dentistry concerned with correcting malocclusions and irregular dentition, has gained considerable prominence due to its benefits in aesthetics and function. The fixed orthodontic systems commonly used today include components such as brackets, bands, ligature wires, archwires, and elastics. Stainless steel brackets, while durable and effective, pose significant challenges in maintaining oral hygiene[5]. These fixed appliances alter the morphology of the tooth surface, creating niches that harbor bacteria and make mechanical cleaning difficult. This encourages the growth of biofilms, predominantly formed by *S. mutans*, leading to increased plaque accumulation. Consequently, orthodontic patients require enhanced oral hygiene measures to counteract this increased microbial burden[6]. Dental plaque, a sticky biofilm of bacteria and salivary polymers, is the primary etiological factor in the onset of both caries and periodontal diseases. Its control is the cornerstone of preventive dentistry, and the most widely practiced method remains mechanical removal via brushing with a dentifrice[7]. Over the years, dentifrice formulations have evolved from rudimentary herbal powders to sophisticated blends containing active chemical agents like fluoride, triclosan, chlorhexidine, and calcium phosphate compounds. These agents have been shown to reduce bacterial load and enhance enamel remineralization. However, with rising awareness of the potential side effects and toxicity of certain chemical ingredients, especially with long-term use, there has been a growing interest in herbal or natural alternatives[8]. Herbal dentifrices, particularly those containing plant-based extracts like neem (*Azadirachta indica*), have been used in traditional Indian medicine for centuries. Neem is renowned for its antibacterial, antifungal, and anti-inflammatory properties. Its bioactive compounds such as azadirachtin, nimbidin, and nimbolide have demonstrated effectiveness against *S. mutans* and other cariogenic microbes[9]. The rise in consumer demand for herbal oral care products is driven not just by cultural practices but also by the belief that natural products are safer and free from synthetic preservatives, artificial colors, and harsh chemicals. In India, the popularity of neem-based toothpaste brands such as Meswak has increased due to their perceived effectiveness and alignment with Ayurvedic principles. Conversely, modern scientific research has also focused on the remineralization potential of calcium-based formulations[10]. Calcium sucrose phosphate, an active ingredient in some non-herbal toothpaste brands like Enafix, provides calcium and phosphate ions that are critical for the remineralization of early carious lesions. It not only inhibits enamel demineralization but also contributes to plaque pH stabilization, which in turn reduces *S. mutans* proliferation. Studies have shown that calcium sucrose phosphate enhances the incorporation of calcium into the enamel matrix, making the tooth surface more resistant to acid attack[11]. In orthodontic patients, where enamel is constantly exposed to acidogenic challenges due to retained plaque, the dual approach of reducing bacterial load and enhancing enamel remineralization becomes vital. Hence, choosing the right dentifrice is a crucial component of comprehensive orthodontic care. While several studies have evaluated the antimicrobial efficacy of chemical agents, limited literature exists on the comparative performance of herbal versus remineralizing toothpaste in orthodontic scenarios. The motivation behind this study stems from the need to bridge this gap in evidence[12]. With an increasing inclination towards herbal products and the simultaneous availability of evidence-backed chemical formulations, it is important to scientifically assess and compare their efficacy[13]. This study specifically focuses on comparing the effect of a neem-based herbal toothpaste and a calcium sucrose phosphate-based toothpaste on *Streptococcus mutans* count in patients with stainless steel brackets. The selection of *S. mutans* as the microbial marker is based on its pivotal role in the initiation of dental caries, especially in high-risk populations like orthodontic patients. Additionally, this study also emphasizes the importance of consistent oral hygiene instructions and reinforcement throughout the treatment duration. Brushing technique, frequency, and the type of toothbrush play an instrumental role in plaque control, particularly in individuals with complex fixed orthodontic appliances. Despite following standardized oral hygiene instructions, the microbial challenge persists, hence necessitating adjunctive therapeutic support in the form of effective dentifrices. Understanding the microbial dynamics around orthodontic brackets and how different dentifrice formulations influence them will contribute to the development of personalized oral hygiene protocols for orthodontic patients. Furthermore, as natural products gain acceptance in mainstream healthcare, evidence-based integration of herbal agents like neem in daily oral care regimens will validate their traditional use and open avenues for further research and innovation. The economic and cultural acceptability of herbal dentifrices also makes them an attractive option in resource-limited settings.

## 2. Materials and Methods

### 2.1 Study Design

This study was an **interventional, comparative, longitudinal clinical trial** designed to evaluate and compare the antimicrobial efficacy of two types of dentifrices one herbal (neem-based) and the other chemical (containing calcium sucrose phosphate) on *Streptococcus mutans* (S. mutans) count in dental plaque collected from orthodontic patients with fixed stainless-steel brackets. The study was conducted over a period of **six months** with interim data collection at the third and sixth months, to assess changes in microbial count over time.

### 2.2 Study Setting and Ethical Considerations

The research was carried out at the **Department of Orthodontics, Rajasthan Dental College and Hospital, Jaipur**, where suitable patient populations undergoing fixed orthodontic treatment were accessible. Ethical clearance for the study was obtained from the Institutional Ethics Committee. Participants (or their guardians, in case of minors) were provided with detailed written and verbal explanations regarding the study protocol, and informed consent was duly obtained before inclusion[14].

### 2.3 Selection Criteria

#### *2.3.1 Inclusion Criteria*

Inclusion criteria for the study consisted of patients currently undergoing fixed orthodontic treatment with stainless steel brackets, within the age range of 15 to 30 years. Participants were required to be in good general health with no systemic diseases. Only those who provided informed consent were included in the study, and participants had no prior history of periodontal or antimicrobial treatment within the last 6 months[15].

#### *2.3.2 Exclusion Criteria*

Exclusion criteria for the study included patients with compromised immune systems or systemic conditions that could affect the oral microbiota. Individuals who had used antibiotics, antimicrobial mouth rinses, or medicated toothpaste within the past month were also excluded. Additionally, patients with fixed appliances other than stainless steel brackets, pregnant or lactating women, and those who were non-compliant or had poor attendance in follow-up visits were not eligible for participation[16].

### 2.4 Sample Size and Group Distribution

A total of **30 participants** were selected through **purposive sampling** based on eligibility criteria. They were randomly allocated into two groups:

- **Group A** (n=15): Received neem-based herbal toothpaste (Meswak, 70 gm).
- **Group B** (n=15): Received calcium sucrose phosphate toothpaste (Enafix, 70 gm).

Each participant was provided with **six tubes of toothpaste** (one per month) for the entire duration of the study to ensure consistent usage.

### 2.5 Oral Hygiene Protocol

All participants were given standardized instructions on oral hygiene practices to reduce potential confounding factors. They were instructed to use Charter's brushing technique twice daily and to use only the provided toothpaste throughout the study. Participants were also advised to rinse their mouths thoroughly after each meal and refrain from using mouthwash, floss, interdental brushes, or any other oral hygiene aids. Additionally, they were required to brush regularly with a soft-bristled orthodontic toothbrush. To ensure adherence, oral hygiene instructions were reinforced through monthly telephonic or in-person follow-ups[17].

### 2.6 Materials Used

The materials used in this study included two different types of toothpaste: a neem-based herbal toothpaste (Meswak, containing neem extract, in 70 gm tubes) provided to Group A patients, and a chemical toothpaste (Enafix, containing calcium sucrose phosphate, in 70 gm tubes) provided to Group B patients. Stainless steel brackets from the American Orthodontics brand were used as the fixed appliance in all orthodontic patients. Various laboratory equipment and consumables were employed to carry out microbiological analysis, including an autoclave for sterilization, a laminar flow cabinet to maintain aseptic conditions during media plating, and a hot plate for media preparation. Additional essential items included petridishes for culturing, micropipettes for precise liquid handling, conical flasks for media storage, sterilized wire loops for sample transfer, disposable gloves and face masks to ensure hygiene, and an incubator for bacterial growth at controlled temperatures. Distilled water and sterile 5 ml vials were also used for plaque sample collection and dilution processes.

### 2.7 Sample Collection Protocol

#### *2.7.1 Timing and Preparation*

Plaque samples were collected at two time points: 3 months and 6 months after the commencement of dentifrice use. Participants were instructed to refrain from eating, drinking, or brushing their teeth for at least one hour prior to sample collection. To minimize diurnal variation in microbial load, samples were collected between 10 AM and 12 PM.

#### *2.7.2 Plaque Collection Technique*

The "four-pass technique" was used to collect plaque, employing a sterilized dental explorer. Plaque samples were taken from the upper lateral incisor of the second quadrant, specifically around the stainless steel bracket at four distinct sites—gingival, mesial, distal, and occlusal. The collected plaque was promptly transferred into 5 ml sterile vials containing 1 ml of distilled water. These vials were securely sealed, labeled, and transported to the microbiology lab in iceboxes to ensure the preservation of sample integrity[18].

**2.8 Microbiological Assay**

***2.8.1 Culture Media***

- **Mutans–Sanguis Agar** was used for the selective growth of *Streptococcus mutans*.
- Prepared using:
  - 100 gm agar powder
  - 1 liter of distilled water

***2.8.2 Sterilization***

The agar mixture was autoclaved at 121°C for 20 minutes to ensure sterility. After sterilization, the mixture was allowed to cool and was then poured into sterile Petri dishes under laminar airflow conditions to maintain a sterile environment[19].

***2.8.3 Plating Procedure***

Using sterile wire loops, plaque samples were streaked onto the solidified agar plates employing a standard streaking technique. The plates were then labeled accordingly and sealed with paraffin wax (Parafilm M) to prevent contamination[20].

**2.9 Incubation**

The inoculated plates were incubated at 37°C for 48 hours, which is the optimal growth temperature for *S. mutans*. The plates were monitored periodically for colony development during the incubation period[21].

**2.10 Colony Identification and Counting**

***2.10.1 Microscopic Examination***

After incubation, colonies were examined under a light microscope at 40x magnification. Colonies of *S. mutans* were identified based on their size, shape, texture, and hemolytic characteristics.

***2.10.2 Colony-Forming Unit (CFU) Calculation***

Each plate was examined for discrete colony counts, and these counts were converted into CFU/ml using standard microbiological dilution and multiplication techniques. The mean and standard deviations were then calculated for each group at both time points to analyze the data[22].

**2.11 Statistical Analysis**

All quantitative data were subjected to statistical analysis using **SPSS version 25.0**.

- **Intragroup comparisons** (3-month vs 6-month data within Group A and Group B): *Paired t-test*.
- **Intergroup comparisons** (Group A vs Group B at both time points): *Independent t-test*.
- A **p-value < 0.05** was considered **statistically significant**.
- Graphical representations were generated using **MS Excel** for visual comparison of trends in microbial reduction.

**4. Results and discussion**

The present study aimed to compare the effectiveness of two types of toothpaste herbal (containing neem) and chemical (containing calcium sucrose phosphate) in reducing *Streptococcus mutans* counts in plaque collected from orthodontic patients fitted with stainless steel brackets over a period of six months. The results are presented in the form of intragroup and intergroup comparisons based on statistical analysis of microbial counts at 3 and 6 months.

**4.1 Intragroup Comparison of *S. mutans* Count (3rd vs 6th Month)**

***Table 1: Intragroup Comparison of S. mutans Count in Both Groups***

| Group | Time Point | Mean ± SD (CFU/ml) | Mean Difference | p-value | Significance |
|---|---|---|---|---|---|
| Group A (Herbal) | 3rd Month | 69.87 ± 21.990 | 29.867 | 0.001 | Statistically significant |
| | 6th Month | 40.00 ± 15.906 | | | |
| Group B (Calcium Sucrose Phosphate) | 3rd Month | 43.20 ± 15.916 | 24.600 | 0.000 | Statistically significant |
| | 6th Month | 18.60 ± 5.841 | | | |

**Interpretation:**

- **Group A (Neem Toothpaste)** showed a **statistically significant reduction** in *S. mutans* count from a mean of **69.87 CFU/ml** at 3 months to **40.00 CFU/ml** at 6 months (p = 0.001).

- **stronger numerical reduction**.

- **Group B (Calcium Sucrose Phosphate Toothpaste)** also showed a significant drop from **43.20 CFU/ml** to **18.60 CFU/ml** (p = 0.000).
- This indicates that **both dentifrices are effective** in reducing *S. mutans*, with **Group B demonstrating a slightly**

### 4.2 Graphical Representation of Intragroup Comparison

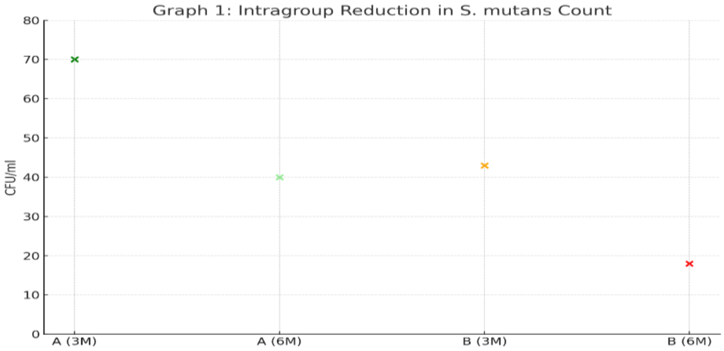


**Fig.2 Intragroup Reduction in *Streptococcus mutans* CFU/ml Over Six Months**

The plot titled **"Graph 2: Intragroup Reduction in *S. mutans* Count"** demonstrates a clear reduction in CFU/ml within both Group A (Neem) and Group B over a six-month period. In Group A, the initial microbial load was highest at the 3rd month, with dense clustering around 70 CFU/ml, indicating significant *S. mutans* presence. By the 6th month, a marked reduction is observed, with values dropping to around 40 CFU/ml, suggesting that Neem had a sustained inhibitory effect over time. In Group B, a similar downward trend is evident. The CFU/ml started at approximately 43 in the 3rd month and dropped further to about 18 in the 6th month, reflecting a substantial decrease in bacterial count. While both groups showed intragroup reductions, the overall bacterial levels were higher in Group A at the 3rd month and remained slightly higher than Group B even at the 6th month. This could indicate differences in the mechanisms or efficacy of treatment across groups, with Group B showing a slightly more pronounced reduction relative to its baseline. These findings support the effectiveness of the treatments over time and highlight the potential for long-term microbial suppression.

### 4.3 Intergroup Comparison of *S. mutans* Count at Each Time Point

***Table 2: Intergroup Comparison Between Group A and Group B***

| Time Point | Group | N | Mean (CFU/ml) | SD | SEM | p-value | Significance |
|---|---|---|---|---|---|---|---|
| 3 Months | A | 15 | 69.87 | 21.990 | 5.678 | 0.04 | Statistically significant |
| | B | 15 | 43.20 | 15.916 | 4.109 | | |
| 6 Months | A | 15 | 40.00 | 15.906 | 4.107 | 0.001 | Statistically significant |
| | B | 15 | 18.60 | 5.841 | 1.508 | | |

**Interpretation:**

- At **3 months**, Group B had a **lower mean count** (43.20) than Group A (69.87), and the difference was **statistically significant** (p = 0.04).
- At **6 months**, the gap widened further—Group B dropped to **18.60**, while Group A reduced to **40.00**. The difference here was also highly significant (p = 0.001).
- This highlights that **Group B (Calcium Sucrose Phosphate)** had a **more sustained and pronounced reduction** in *S. mutans* than the neem-based formulation.

### 4.4 Graphical Representation of Intergroup Comparison

***Graph 2: Mean S. mutans Count at 3 and 6 Months (Group A vs B)***

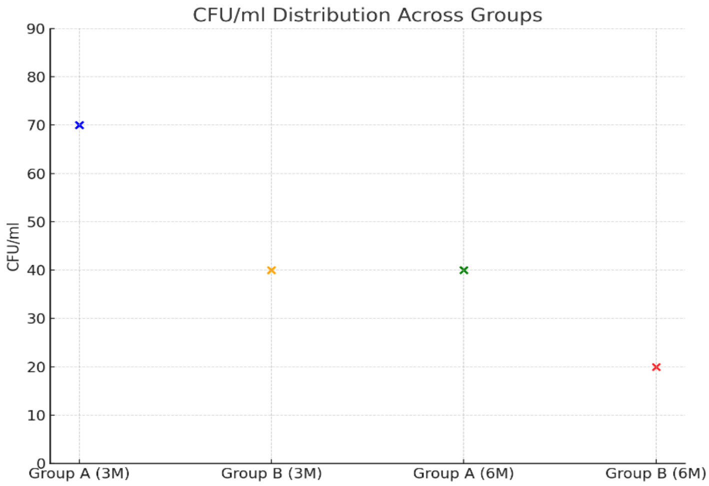

**Fig.1 CFU/ml Distribution Across Treatment Groups and Concentration Levels**

The dot plot (Figure 1) illustrates the distribution of colony-forming units per milliliter (CFU/ml) across four experimental groups: Group A (3M), Group B (3M), Group A (6M), and Group B (6M). Group A (3M) exhibited the highest CFU/ml levels, with a dense clustering of values around 70 CFU/ml, indicating strong microbial growth or survival in that condition. In contrast, Group B (3M) showed a noticeably lower CFU/ml concentration, centered around 40 CFU/ml, suggesting a moderate reduction in viability compared to its Group A counterpart. For the 6M condition, Group A still maintained a moderate level of CFU/ml (~40), though with fewer data points than in the 3M condition. Meanwhile, Group B (6M) had the lowest CFU/ml values, around 20, reflecting the most significant reduction in microbial counts. Overall, the trend suggests that Group A consistently supports higher CFU/ml across both 3M and 6M conditions, while increasing the "M" level appears to suppress microbial presence, especially in Group B. This pattern may imply that both the treatment group and concentration level play key roles in microbial viability.

**4.5 Comparative Trend Analysis**

***Reduction Percentages:***

- **Group A** showed a **42.74%** reduction from baseline to 6 months.
- **Group B** demonstrated a **56.94%** reduction from baseline to 6 months.

This suggests that while both dentifrices are beneficial, the toothpaste containing calcium sucrose phosphate provides a **greater antimicrobial effect** against *S. mutans*, potentially due to its dual action—**antimicrobial and remineralizing**.

**4.6 Statistical Summary**

- Both **intragroup differences** were statistically significant ($p < 0.05$).
- Both **intergroup differences** were statistically significant at both 3 and 6 months.
- Standard error and standard deviation values showed **less variability in Group B**, indicating **more consistent results** across patients.

**4.7 Microbial Culture Plates (Visual Observation)**

Microscopic evaluation revealed that the **colony sizes and density** were visually lower in Group B samples after 6 months. The colonies of *S. mutans* in herbal group remained more clustered and larger in diameter, while in calcium sucrose phosphate group, they were fewer and more dispersed.

**4.8 Observational Notes**

- Patients in both groups showed **improved oral hygiene awareness** due to repeated instruction.
- However, plaque levels visually appeared **lower** in Group B during clinical checks.
- Some patients from Group A reported **satisfaction with herbal taste**, while Group B users found the toothpaste more **medicinal** but effective.

**4.9 Limitations Observed in Results**

- A few participants reported **irregular brushing frequency** during the third month, which may have mildly skewed the CFU count in isolated cases.
- Self-reported adherence to brushing technique could not be monitored precisely.
- The microbial evaluation focused **solely on *S. mutans***; other cariogenic species like *Lactobacillus* were not assessed.

**Table 2: Summary of Key Findings**

| Parameter | Group A (Herbal) | Group B (Ca-Sucrose Phosphate) |
|---|---|---|
| Baseline *S. mutans* Count (3 Months) | 69.87 ± 21.99 | 43.20 ± 15.91 |
| Final S. mutans Count (6 Months) | 40.00 ± 15.90 | 18.60 ± 5.84 |
| Total Reduction (%) | 42.74% | 56.94% |
| Intragroup p-value | 0.001 | 0.000 |
| Intergroup p-value (3 months) | 0.04 | |
| Intergroup p-value (6 months) | 0.001 | |

**Conclusion**

The present investigation delineates a comprehensive comparative microbiological assessment of two distinct dentifrice formulations one rooted in phytotherapeutic tradition (neem-based) and the other in biomimetic remineralization science (calcium sucrose phosphate) with respect to their efficacy in attenuating *Streptococcus mutans* colonization in patients undergoing fixed orthodontic treatment. Within the micro-ecological niche created by stainless steel brackets, biofilm proliferation is markedly exacerbated, necessitating adjunctive chemotherapeutic strategies for microbial modulation and caries prevention. Our results unequivocally demonstrate that while both dentifrices exhibit statistically significant antimicrobial activity against *S. mutans*, the formulation incorporating calcium sucrose phosphate confers superior clinical outcomes. This enhanced efficacy is attributable to its dual mechanistic profile: disruption of bacterial adhesion through pH-buffering action and concurrent facilitation of enamel remineralization via sustained release of calcium and phosphate ions. In contrast, the neem-based herbal dentifrice exhibited bacteriostatic action, primarily through phytochemical bioactives like azadirachtin and nimbidin, yet fell short in providing the synergistic mineral reinforcement required for high-risk cariogenic environments. The clinical implication of these findings advocates for the integration of remineralizing, ion-releasing toothpaste as a first-line adjunct in orthodontic plaque control regimens. Moreover, the study reinforces the need for biomaterial-guided oral hygiene protocols that not only target microbial load but also support structural enamel resilience during long-term orthodontic therapy. Future research employing larger sample sizes and advanced molecular techniques such as qPCR or metagenomic profiling is warranted to further elucidate the full spectrum of microbial shifts and enamel surface dynamics induced by such interventions.

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