Comparative Evaluation of Antimicrobial Efficacy of Toothpastes Containing Probiotic and Neem as Primary Ingredient on Salivary *Streptococcus mutans* in Melmaruvathur Population: An *In Vivo* Study

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**Aim:** This *in vivo* study was designed to compare the antimicrobial effect of toothpastes containing probiotics and neem on *Streptococcus mutans* in 18–30-year-old patients in Melmaruvathur population.

**Materials and Methods:** The study consisted of 60 patients who were randomly divided into two groups of 30 each. Group I received probiotic-based toothpaste (PerioBiotic), whereas Group II received neem-based toothpaste (Babool) as a preventive measure protocols to control the incidence and prevalence of dental caries.

**Study Design Protocols:** Participants were instructed to use the dentifrice selected for the study, two times a day for 60 days. Tests were performed on the saliva samples at the beginning of the study, 0 day, 15th day, 30th day, and 60th day following the use of toothpaste. The Statistical Package for the Social Sciences (SPSS-25) software, version, and Chi-square and one-way analysis of variance were used for data analysis.

**Results:** The toothpastes containing neem and probiotics as primary ingredients were efficient in reducing the number of bacterial count when comparing the baseline data with the 60 days data in both the groups as a therapeutic regimen. Intragroup values showed reduction in the number of bacterial count in both the groups in a gradual manner, whereas the intergroup values between the toothpaste showed no statistically significant difference in the bacterial count, and both toothpastes were efficient in reducing the bacterial count.

**Conclusion:** This study reveals the influence of probiotics and neem on salivary *S. mutans* levels. The results revealed that neem-based and probiotic-based toothpaste, which were tested in this study, showed comparable antibacterial activity against the *S. mutans*. The neem-based toothpaste showed promising and good antimicrobial activity and reduced the level of bacterial count, which can be attributed to the presence of secondary metabolites, such as flavonoids, alkaloids, lectins, and polyphenols, as its active components. The effect of each component against the *S. mutans* needs to be quantified in further studies, and within the limitation of this short study, it can be considered as a better choice than commercially available toothpaste, which predominantly has chemicals as active ingredients, which in turn can compromise the safety aspects in the patients when used for controlling the dental caries for a longer duration.

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INTRODUCTION

Dental caries is a multifactorial disease, which occurs due to the contribution from the various factors, among which, the host factors play a key role along with the bacterial, dietary, and time factors, which are responsible for the incidence of caries. [1]

In the host factor, the difference in morphology and arrangement and location of teeth contributes to the initiation of caries by forming trap areas for the bacteria to get colonized in the plaque and turns down as a biofilm, which is a natural process.

The saliva is one of the important host factor, which has a natural major role in prevention of dental caries due to its innate antibacterial activity exhibited due to presence of salivary immunoglobulin, which inhibits the bacterial attachment into tooth surface.

The other salivary factors, including flow rate, viscosity, composition, and pH, all contribute to the anticarious activity.

Alteration in the composition of saliva, which continuously comes in contact with the tooth surface will be the primary target to effectively control the caries.

Caries activity is a dynamic one with active demineralization and static phases that coexist, and it occurs due to the imbalance between the loss and gain of the minerals. [1] Dental caries is initiated under the influence of Streptococcus mutans, a predominant organism isolated in the saliva of carious patients. [2]

The cariogenic potential of S. mutans is related to the virulence factors, mainly adhesion capacity, acidogenicity, and aciduricity, which are responsible for the enamel caries in initiation and progression; in later stages, the Lactobacillus is responsible for the progression in dentinal caries. [3,4]

It has been a real challenge for the dental community to control the aforementioned disease as early as possible to avoid a major complication, if it is left unnoticed and untreated, the simple white spot lesion can turn into a life-threatening cellulitis.

The focus now shifts toward the implementation of the preventive measures, such as brushing and flossing, which are still now the golden standard measures in controlling the plaque directly, and indirectly in the prevention of dental caries. [5]

Various innovations in material science have made us to include probiotics as active ingredients in toothpastes and mouthrinses, as these methods have significant effects in oral health and in preventing dental caries.

Probiotics are living organisms, which in sufficient amount, provide health benefits. [6] Lactobacillus paracasei is a probiotic microorganism strain, which is marketed as PerioBiotic toothpaste, a therapeutic innovation against the war on dental caries; [7,8] the proposed action is that it binds and eliminates salivary microbial counts of S. mutans present in the saliva.

The active constituents of herbal toothpastes are supposed to have antibacterial property but they are not substantiated by the dental literature.

The inclusion of neem is one in that direction, which has a diverse action in the maintenance of oral ecology. [9]

There are many other contributing factors that also play a key role in the prevention of dental caries, which include the host factors, dietary factors time, frequency, and type of carbohydrate play a key role in the progression of dental caries. For an effective prevention and control of carious process, the viscous cycle has to be targeted.

Aim: The aim of this study was to compare the antimicrobial effects of probiotic- and neem-based toothpaste on levels of S. mutans in the saliva of patients with high-to-moderate risk for dental caries on population in and around Melmaruvathur. This is an in vitro study.

MATERIALS AND METHODS

Inclusion criteria

Patients with normal to low caries risk are ideal for preventive measure assessment. Patients with moderate to high caries risk are ideal for therapeutic benefit assessment.

Exclusion criteria

Patients with no history of antibiotic or antimicrobial agent usage within the last month, and people using dairy probiotics regularly were included in exclusion criteria.
The study protocol was approved by the ethics committee of APDCH. All volunteers were asked to read the informed consent form, and to sign it if they agreed to its contents. The participants were randomly divided into two groups (each comprising 30 patients): Group I—probiotic toothpaste (PerioBiotic, Designs for Health, Arlee, Montana) and Group II—neem-based toothpaste (Babool, Dabur Pvt Ltd, New Delhi).

**Standardization**

Patients were provided with toothbrushes of medium hardness and were instructed to brush their teeth thoroughly two times per day for 2–3 min using their allocated toothpastes and a modified bass brushing technique. During the study duration, the participants were restrained from using any other toothpaste or oral product from another supplier, and were instructed to follow the same oral hygiene habits regularly.

**Isolation and characterization of Streptococcus mutans**

The salivary samples from patients with high-to-low risk dental caries were collected at the Department of Conservative Dentistry, Adhiparasakthi Dental College and Hospitals, Melmaruvathur, Tamil Nadu, India.

The samples were brought to laboratory in transport fluid medium (0.4% agar, 0.15% thioglycolate/phosphate-buffered saline).

The levels of *S. mutans* were evaluated by CRT bacteria (Vivadent, Schaan, Liechtenstein) in department of microbiology, Melmaruvathur Adhiparasakthi institute of Medical Sciences, at the baseline and after 15, 30, and 60 days of intervention.[14]

The participants were advised to avoid any solid and liquid diet for 2 h before collecting saliva and were instructed to brush their teeth morning only on the day of the sample collection. A total of 2 mL of stimulated saliva was collected into a sterile plastic cup for 5 min during paraffin-wax chewing.

The collected saliva sample was transferred to an appropriate culture medium in glass tubes and was incubated at 37°C for 48 h. After incubation, the score of colony forming units was obtained by comparing the test strip with an evaluation chart provided by the manufacturer.

**RESULTS**

2. Chi-square test for *S. mutans* count assessment before and after usage for 60 days for Group II (neem)

| S. no. | Days | Less than $10^5$ | Greater than or equal to $10^5$ |
|--------|------|-----------------|-------------------------------|
| 1      | 0    | 9               | 15                            |
| 2      | 15   | 6               | 10                            |
| 3      | 30   | 5               | 7                             |
| 4      | 60   | 3               | 4                             |

All the study participants stated that they brushed their teeth by using the toothpastes given to them for 60 days. Tables 1 and 2 present the pretreatment and posttreatment levels of *S. mutans* in saliva. Majority of the participants had high counts of salivary *S. mutans* at baseline.

After the 60 days use of probiotic-based toothpaste, and neem containing tooth paste the number of *S. mutans* decreased. In addition, an important decrease was recorded in the participants with the *S. mutans* percentage using the neem-based toothpaste for 60 days.

The results obtained in our study have shown that if the PerioBiotic probiotic-based toothpaste and neem-based toothpaste are used twice a day, a decrease in the number of the bacteria in the saliva was observed after 60 days.

The toothpastes containing neem and probiotics as the primary ingredients were efficient in reducing the number of bacteria when comparing the baseline data with the 60 days data in both the group as a therapeutic regimen.

Intragroup values showed reduction in the number of bacteria in both the groups in a gradual manner, whereas in the intergroup values between the toothpaste, no statistically significant difference was observed in the bacterial count, and both toothpastes were efficacious in reducing the bacterial count.

**DISCUSSION**

This study has been performed to determine whether the two potential antimicrobial agents, namely
probiotic bacteria–based toothpaste and neem-based toothpaste would improve the suppressive effect on bacteria responsible for caries initiation in the saliva.

The levels of S. mutans were evaluated by CRT bacteria (Vivadent) in department of microbiology, MAPIMS, at the baseline and after 15, 30, and 60 days of intervention. The patients were instructed to chew a paraffin pellet for the purpose of transfer of the bacteria from the tooth surfaces to the saliva. Then saliva was collected in a container. It is also necessary to check the salivary flow rate and the buffering capacity with CRT buffer from Ivoclar Vivadent.[10] Sodium bicarbonate tablet placed in the test vial releases CO₂ when it comes into contact with moisture. This creates favorable conditions for bacterial growth. After the protective foil has been removed, working process should be fast.

Agar should not be left open for extended periods. Sneezing or coughing near the agars must be avoided.

Agar should be entirely covered with saliva using a pipette. During this process, care should be taken to avoid scratching the culture media.

Bacteria will only grow in areas, which have come into contact with saliva. The carrier should be placed in oblique direction to prevent the rapid flow of saliva and also to help for thorough wetting of the surface. The agar carrier is immediately placed in the test vial, which is tightly sealed. Then it is placed in an incubator at 37°C/99°F to allow the growth of bacterial colonies. Even when the bacteria are placed for more than 2 days, it does not cause any problems. For the purpose of demonstration, test samples can be stored in the refrigerator for up to 2 weeks. Agar carriers were discarded after they were cleaned with a suitable disinfectant or autoclaving.[11]

According to Prabhuswamy et al.,[24] there are many factors related to microbes, which have to be breached to exert an antimicrobial action, which are: (1) nutrients as component of its cell structure, (2) water content, (3) oxygen availability, and (4) temperature. The concepts of microbes living in complex niche as biofilm make the bacteria eradication a tough task, the reasons for the aforementioned are: (1) irreversible clustering of bacterial cells hooked up onto tooth surface entombed in an exopolymeric matrix and (2) resistant to physical and shear forces created by washing action of saliva and phagocytosis.

In this study, S. mutans was used as it is the principle etiological agent of dental caries. After the establishment of primary colonizers, they invite secondary colonizers with series of changes in oral ecology, contributing to the formation of biological niche, causing cavitations as end outcome if it is left undisphd. Streptococci Mutans occur as small blue colonies with a diameter of less than 1 mm on the blue agar, whereas lactobacilli grow as white colonies on the transparent agar.[12]

If the number of mutans Streptococci and/or Lactobacilli per milliliter of saliva is more than 10⁵ CFU, then it indicates a high risk.

If the bacterial counts are high in any case, then it is an indicator of a high caries risk, that is, latent risk of developing caries.[13] Given the nature of caries, however, no reliable, general forecast can be made by examining only one etiological factor.[15] Therefore, caries does not necessarily develop even few protective factors are present. However, such as reduced intake of fluoride or the increased intake of sugar, caries will develop (Brathall, 1996).

Hence, new caries lesions will develop if high bacterial counts have been recorded (Kristofferson et al., 1985). An early control of the bacterial counts may contribute to a decrease in caries development in the long run, as corresponding measures can be introduced.[16]

According to the definitions of the World Health Organization (WHO) and the American Food and Agricultural Organization (FAO), there are many microorganisms that are called probiotics; Lactobacillus and Bifidobacterium are common probiotics.

Bacterial resistance against antibiotics is one of the major shortcomings against the usage of antibiotic-based ingredients in it so the idea of using probiotic treatment for oral health has emerged.[20] Probiotics and their effect on oral health have been a focus of numerous trials in recent times. No documented trials have been reported with its focus on probiotics as toothpaste, especially in the Melmaruvathur population.

Many clinical studies have showed that the regular consumption of milk and cheese, which contain probiotics, leads to the decrease in the number of the cariogenic Streptococci in the saliva and dental plaque.[21,22]

A previous study by loesche et al. stated that the regular usage of yogurt containing Lactobacillus reuteri for more than 2 weeks led to a decrease of more than 80% in the S. mutans concentration in the saliva. Mestrovic et al.[21] stated that probiotic-based toothpaste that contained L. paracasei probiotic strains led to a reduction of S. mutans in the saliva, the L. paracasei
has an advantage in terms of requiring 10 s in the oral cavity to become active.\textsuperscript{[22]}

\textit{L. paracasei} mouthrinse has a effect of 50\% reduction in the \textit{S. mutans} bacteria.\textsuperscript{[24]} The major limitation in the usage of probiotics, according to previous literature, as an effective antimicrobial agent is on the existence of less literature evidence on the proposed antimicrobial mechanism.

This study was designed to understand the mechanism of action with the help of recent advances in the molecular studies for establishing the exact nature of antibacterial action and its efficacy; the other factors rely on (1) form of usage, (2) dose, (3) frequency, and (4) transport mechanism to the target area, which in this study was to reach the saliva effectively.\textsuperscript{[23]}

The success of plaque control measures relies predominantly on patient-related factors and toothbrushing, which is predominantly mechanical maneuver used for the maintenance of oral hygiene.\textsuperscript{[17]}

The practice of oral hygiene measures as well as the selection of dentifrices with antibacterial efficacy plays a major role in caries prevention protocols.\textsuperscript{[30]} The second key factor in the toothbrushing procedure relies on the usage of toothpaste with many active ingredients that were predominantly chemicals used for diverse actions.

Recently, there is a paradigm shift in the usage of ingredients with emphasis placed more on the efficiency with minimal effects to the patients, insisting more on the safety aspects of the ingredients used in the toothpaste, which has made us to move toward the usage of herbal-based ingredients.\textsuperscript{[35]} Natural materials for harvesting therapeutic benefits have undergone changes to: (1) Reduce the bacterial numbers and attachment in caries prevention regimens. It is important to verify the efficacy of any product by clinical or \textit{in vitro} trials, instead of simply accepting the claims made by the manufacturer. The antibacterial efficacy is based on the presence of bioactive compounds capable of causing disruption of the bacterial cell wall, which is predominantly a barrier for the agents. (2) Inhibition of bacterial enzymatic activity, which is essential for the survival of bacteria through the disruption of glycolytic pathways needed for energy production. (3) Reduction of bacterial attachments to the tooth surface by deposition of material, which makes them nonsticky to the tooth surface.\textsuperscript{[26]}

In this study, it was observed that the two tested toothpastes showed significant inhibitory activity against the cariogenic bacteria, \textit{S. mutans}. The antimicrobial activity of the neem-based toothpaste can be attributed to the presence of secondary metabolites, such as alkaloids, flavonoids, polyphenols, and lectins, as observed in the previous studies.\textsuperscript{[24-32]}

In future studies, the exact mechanism of actions needs to be explored by designing studies in such a way so as to harvest the anticarious benefits. Among all the tested toothpastes, neem-based toothpastes were found to be more effective compared to other toothpastes in accordance with previous work by Caglar \textit{et al.}.\textsuperscript{[19]}

From the composition aspects, the type, number of ingredients, and concentration of each individual components play a key role in the antimicrobial aspect of the toothpaste.\textsuperscript{[19]}

Dentifrice with fewer herbal ingredients had deprived inhibitory effects against cariogenic bacteria compared to one with combination of herbs, which had better effect in inhibiting cariogenic bacteria, which is also confirmed in our study.

This variation may be because (1) solvent of extraction and solvent concentration may be different, (2) concentration of the active compound in the crude extract may be different, and (3) there might be inhibition of active compound by other ingredients. The major limitations of studies were related to the duration of study and the follow-up of patients.

The next major factor is the concentrations of neem used and the form of usage of the ingredients. Water-soluble extracts of neem have property of reducing the adhesion and colonization potential of the bacteria which might me related to change in the surface energy of the tooth structure.\textsuperscript{[19]}

According to Patil \textit{et al.},\textsuperscript{[32]} various factors that have to be taken care of during the delivery of neem as an active ingredient are: (1) freshness of the extracts used in the composition, (2) follow-up period to monitor the effect of stabilization on the bacterial count, and (3) development of potential for bacterial resistance to neem-based products.

**CONCLUSION**

This study was aimed to reveal the influence of probiotics and neem on salivary \textit{S. mutans} and to imply that both neem-based and probiotic-based toothpaste that was tested in this study showed comparable antibacterial activity against the \textit{S. mutans}. The neem-based toothpaste showed promising results and good antimicrobial activity in terms of reduction in the level of bacteria.

This can be attributed to the presence of secondary metabolites, such as alkaloids, flavonoids, polyphenols,
and lectins, and it is a better choice than the commercially available toothpaste, which has chemicals as active ingredients and can compromise the safety effects if it is used for a longer duration. Herbal toothpastes significantly inhibit the \textit{S. mutans}, especially the neem-based toothpaste have the prospect of becoming safer and effective alternative and provide an ideal home care regime. It could be recommended as therapeutic toothpaste, especially in high risk for caries.

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**Conflicts of interest**

There are no conflicts of interest.

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