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Clinical estimation of anticaries effect of probiotic toothpaste among 18-25 years old young adults- A double blinded randomized controlled trial

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Abstract

Introduction : The aim and objective of the study was to estimate the anticaries effect of probiotic toothpaste by assessing the Streptococcus mutans and Lactobacillus levels and by recording indices before and after intervention among 18-25 years old young adults.

Materials and Methods : A double blinded, randomized controlled trial was carried out among thirty healthy volunteers of young adults in the city of Madurai. The subjects were randomly divided into two equal groups of A and B. At baseline, samples of saliva were collected to check Streptococcus mutans and Lactobacillus levels and indices like Oral Hygiene Index (1960), Modified Turesky Plaque Index (1970), Stain Index (1968) and Gingival Index (1963) were recoded. Group A received Conventional toothpastes (Colgate cavity protection) and Group B received Probiotic toothpastes (Purexa). For the next 15 days, they were instructed to brush using only the allotted toothpastes. The saliva sample collection and recording of indices were repeated after 15 days post intervention.

Results : Gingival and oral hygiene indices showed significant differences of mean in Group B (0.14, 0.28; $p<0.05$ respectively). A significant difference of means was noted between Group A and B in the case of Plaque, Stain, Oral Hygiene indices and the bacterial count (Lactobacilli and Streptococcus mutans) (A :0.10, -0.12, 0.01; B :0.06, 0.09, 0.28 and -12.8, -14.8 ; $p<0.05$ respectively).

Conclusion : The probiotic toothpastes illustrated a significant reduction of Lactobacillus, Streptococcus mutans bacterial count, Stain, Plaque and Oral Hygiene index scores compared to conventional toothpastes.

Key words : probiotic, anticaries, toothpaste, adults

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INTRODUCTION

Most dental health issues can be avoided or treated when they are still at their initial stages. Dental caries, periodontal diseases, oral cancers, oro-dental trauma, cleft lip and palate, and noma make up most cases (1). According to 2019 Global Burden of Disease report, about 3.5 billion people worldwide suffer from oral disorders, with caries of the permanent teeth being the most common ailment. Around the world, 520 million children and 2 billion adults are thought to be affected by caries of the permanent teeth (2). In systematic review and meta-analysis study of Pragya et al (2021) in India, it was found that the prevalence of dental caries was 54.16 percent overall, 62 percent in patients over the age of 18 years, and 52 percent in patients between the ages of 3 and 18 years. Prevalence rates were highest overall in mixed dentition (58 percent) (3).

A healthy adult's oral cavity is home to about 400 types of commensal microorganisms (4). Increased cariogenic bacteria result from an anomaly in this ecology caused by dietary practices, poor dental hygiene, or systemic causes (5). Lactic acid is formed when the dietary carbohydrates are broken down by the microorganisms in tooth plaque, which leads to the eventual development of dental caries (6).

Regarding the modern ideas of dental diseases from oral microbial communications, a significant view has opened doors to a innovative method for controlling dental caries via curbing the oral microbial ecology that suggests the selective inhibition of oral pathogens of dental plaque in order to control microbial pathogenesis. This approach was introduced at the commencement of the 20th century by Elie Metchnichkoff, a Russian Nobel Prize laureate, who revolutionarily discovered probiotics which are defined as “live microbial food supplements which beneficially affect the host animal by improving its intestinal microbial balance”. They competitively inhibit only the pathogens due to the greater affinity for tissues. Once the pathogens are swapped, the re-establishment of them is tough (7).

Probiotics are usually taken as a part of diet in some cultures in the form of fermented foods like yogurt, or as dietary supplements with added active live cultures. They have shown potential in the management of a variety of conditions, including malnutrition, lactose intolerance, calcium availability, bowel issues like constipation, urogenital infections, and atopic diseases like antibiotic-induced diarrhoea, assurance in boosting immunity, reducing the symptoms of chronic intestinal inflammatory diseases, and preventing and treating diarrhoea caused by pathogens (8, 9).

Since plaque-induced caries is a local disease, the local use of antimicrobial agents will be effective than their systemic use (10). To maintain good oral hygiene for life, a variety of methods and practices have been developed to reduce bacterial colony numbers and remove plaque. Brushing our teeth is one of them. Dentifrices are the most practical means of delivering antibacterial agents on a regular basis because brushing is thought to be the most popular oral hygiene technique. The goal of these treatments is to prevent dental caries and gingivitis (11). Many probiotic agents like mouthwashes (12), lozenges (13), tablets, straws (14), milk (15), cheese (16), ice cream (17), chewing gums (18,19), yogurt (20, 21) and other supplements has had positive outcomes on dental well-being. Probiotics have been incorporated into mouthwashes and dentifrices for popular consumption since the benefits on oral health in preventing gingivitis, halitosis (22, 23) and caries (20, 21) have been recognized.

With the views of above, the present study was steered with a goal to estimate the anticaries effect of probiotic toothpaste clinically and microbiologically among 18-25 years old young adults in the city of Madurai.

MATERIALS AND METHODS

Study design: The present study is a Double Blinded, Randomized Controlled Trial, designed to estimate the anticaries effect of probiotic toothpastes among young adults of Madurai city.

Study area: Madurai, Tamil Nadu.

Ethical clearance: Authorization was obtained from the Head of the Institution of Best Dental Science college, Madurai to conduct the study among the college students, before starting the study. Ethical approval was gotten from the Institutional Ethical Committee. The method and purpose of the study was thoroughly explained to the subjects. Written informed consent in the study proforma was procured from the subjects before the commencement of the study. It was stressed that discretion would be followed and that no personal details would be involved in the study's report.

Sample size estimation: Sample size was calculated using a priori power analysis from G* Power version 3.1.9.2 software with power as 80% and alpha as 5%. The effect size was calculated from a previously published study (24). The total sample size was calculated to be as 28 and rounded off to 30 and was split equally for two groups.

Eligibility criteria: Study subjects who volunteered to participate within the age of 18-25 years and who were willing to abide by the instructions that was given during the course of the study were included in the study. Subjects who have received antibiotics or antimicrobial agents 3 weeks prior to sample collection and individuals with habit of taking dairy probiotics / xylitol chewing gums & recent topical fluoride treatments; Subjects who had recent dental extraction procedure and who was under Orthodontic treatment were not included in the study.

Blinding, randomization and allocation concealment: Simple random sampling method was followed. The study design is double blinded. Eligible participants were randomly allocated using chit method. The participants were asked to blindly pick any of the two 15 sets of closed chits marked A and B and allotted into two different groups- Group A: Colgate© cavity protection toothpaste and Group B: Purexa© Probiotic toothpaste by a third individual who was not a part of the current study.

The toothpastes were packed in an opaque container and were distributed in similar looking tubes. The palatability of the toothpastes was checked priorly by the investigator in case of uneasiness to the study subjects. The subjects were instructed to only use the allocated toothpastes to brush their teeth for the next 15 days. They were reminded for the same consistently through phone calls and messages for every other day.

Study proforma: A study proforma in English Language was prepared to collect the required data, which had three sections:

1. The first section had informed consent of the subjects and the provision to record participant’s age, gender and signature

2. The second section had provision to record pre and post sample of salivary bacterial load

3. The third section had provision to record clinical examination scoring of the indices

Sample collection and clinical examination: The unstimulated saliva sample was collected from the study subjects in the morning, two hours after breakfast. Subjects were asked to spit 2 milliliters of saliva in a sterile sialometry tube (24,25) which were then transferred to a cold chain box. The box was then transported to the microbiological lab for further investigation on the growth of bacteria. After saliva sampling have been done and stored, subjects were clinically assessed through the following indices based on the original authors’ proposal: Oral Hygiene Index (John C. Greene, Jack R. Vermillion 1960) (26), Modified Turesky Plaque

Index (Quigley & Hein 1962 and modified by Turesky, Glickman, Gilmore 1970) (27), Stain Index (Lobene 1968) (28) and Gingival Index (Löe and Sillness, 1963) (29).

Laboratory procedures: Preparation of MRS media- 67.15 grams of the MRS agar-M641 media was suspended in 1000ml distilled water. It was heated to completely dissolve the medium, until it boiled. The medium was autoclaved for 15 minutes at 121°C and 15 lbs of pressure. After mixing the medium thoroughly, the contents were then transferred to sterilised petri dishes.

Preparation of 48 hours culture- To inoculate the media with sample, the inoculated loops of diameter 5mm were sterilized using 5ml phosphate-buffered saline solution at pH 7.2 at 37oC. The sample was incubated using the loop in the media for 48 hours at 35-37oC in ambient air.

Preparation of the blood agar media- 40 grams of nutrient agar was suspended in 1000 ml purified/ distilled water. To completely dissolve the medium, it was heated until it boiled. By autoclaving for 15 minutes at 121ºC and 15 pounds of pressure, the medium was sterilised. Aseptically 5% v/v sterile defibrinated blood was added after cooling to 45–50ºC. The contents were mixed thoroughly, then transferred to sterilised petri dishes.

Preparation of 24 hours culture- For inoculation of the sample into the Streptococcus mutans- Blood agar media, the inoculated loops were sterilized using 5ml phosphate-buffered saline solution (PBS, pH 7.2) at 37oC under aseptic conditions and the specimens were streaked onto the Blood Agar using the inoculated loops and incubated for 24 hours. The salivary samples were transported to the microbiological lab and incubated accordingly in both the medias and the growth of the bacteria was identified and confirmed using the subsequent confirmatory biochemical tests like Esculin Hydrolysis, Oxidase and Catalase tests for Streptococcus mutans and Gram staining for Lactobacilli and Streptococcus mutans:

Esculin hydrolysis test- Esculin hydrolysis is an effective test for identifying gram-positive and gram-negative bacteria that includes a wide range of facultative anaerobes, aerobes, and anaerobes. An inoculum was obtained from an 18 to 24-hour culture with a sterilized inoculating needle from the centre of an isolated colony. The esculin agar glass vials were inoculated by streaking its surface with the inoculum picked from the culture plate. The inoculated glass vials are then incubated at 35-37°C for 24 hours, and the color change is observed, where black color change of the medium establishes a positive test in the esculin medium with ferric ammonium citrate. No color change shows a negative tube test and ideally Streptococcus species gives negative result which was the case in the current study, confirming the presence of Streptococcus species in the sample (30).

Oxidase test- This test checks for the presence of cytochrome oxidase, also known as indophenol oxidase. Procedure- One or two drops of 1% Kovacs oxidase reagent was placed on the organisms in the agar and the color of it was observed. The microorganisms are oxidase positive if color changes to dark purple within 5-10 seconds. In the current study the color wasn't changed even after 2 minutes, confirming the presence of Streptococcus mutans (31).

Catalase test -This test is used to differentiate those bacteria that produces an enzyme catalase, such as Staphylococci, from non-catalase producing bacteria such as Streptococci (32). A petri dish was placed under the microscope. Using a sterile inoculating loop, a streak of organism was collected from a well-isolated 18 to 24 hours colony and place in the microscope slide. A dropper or pipette was used to place a drop of 3% hydrogen peroxide onto the organism on the microscope slide. The petri dish was immediately covered and observed for any bubbles over the slide. No bubbles were present, indicating a negative result of the test, confirming the presence of Streptococcus mutans (33). The samples were incubated and the developed

colonies were counted. The number of colonies were calculated according to dilution ratio and defined as the number of colony forming units (CFU) per millilitre. The same procedures of saliva sample collection, recording of the indices and laboratory procedures were repeated after a time period of 15 days post intervention

Statistical analysis

Statistics was done using IBM SPSS Statistics 20 package. Paired t test was used in comparing the means of pre- and post- intervention of the indices inside the groups. Independent t test was used in comparing the means of pre- and post- intervention of the bacterial count and the indices between the groups.

RESULTS

The present double blinded, randomized controlled trial checked the anticaries effect of probiotic toothpaste microbiologically in comparison with a commercially available, commonly used toothpaste. Those who were eligible to the (n=30) inclusion criteria were allotted randomly into two groups of intervention with 15 subjects per group. The study subjects comprised of 12 males and 18 females. All the subjects were followed up from the start till the end of the study. 80% was fixed as the power of the study. There was a statistically significant reduction of both the Streptococcus mutans (p=0.000) and the Lactobacilli (p=0.001) counts in Group B (Table 1). Furthermore, there was a statistically significant reduction of scores of Plaque Index in Group A (p=0.005) and Gingival (p=0.02), Oral Hygiene Index (p=0.02) in Group B (Table 2).
There were statistically significant differences of mean of both the Streptococcus mutans (p=0.004) and the Lactobacilli (p=0.006) counts (Table 3). There were statistically significant differences of mean of scores of Stain Index (p=0.02) and Oral Hygiene index (p=0.02) (Table 4).

Table 1: Paired group comparison of Colony Forming Units of both the bacteria - Baseline and post intervention

Variables	Mean	Standard Deviation	95% confidence interval of difference Lower	Upper	Sig. p value
GROUP A (LB) PRE	40.87	20.42	-5.36	6.43	0.849
POST	40.33	19.53			
GROUP B (LB) PRE	44.07	24.02	6.19	20.60	0.001*
POST	30.67	16.7			
GROUP A (SM) PRE	47.00	20.50	-4.12	8.52	0.468
POST	44.80	20.06			
GROUP B (SM) PRE	40.80	18.57	9.15	24.84	0.000*
POST	23.80	8.26			

(Group A- Colgate anticavity, Group B- Purexa toothpaste, LB- Lactobacilli, SM- Streptococcus mutans; PRE: pre-intervention, POST: post-intervention) $p<0.05$, Statistically significant*

Table 2: Intra group comparison of indices within the 2 groups at Baseline and post intervention

Variables	Mean	Standard Deviation	95% confidence interval of the difference Lower	Upper	Sig. p value
GROUP A (SI) PRE	0.06	0.07	-0.27	0.03	0.12
POST	0.18				
GROUP A (PI) PRE	0.31	0.03	0.03	0.17	0.005*
POST	0.20				
GROUP A (GI) PRE	0.57	0.04	-0.01	0.16	0.094
POST	0.50				
GROUP A (OHI) PRE	0.89	0.02	-0.03	0.05	0.546
POST	0.88				
GROUP B (SI) PRE	0.18	0.04	-0.00	0.19	0.058
POST	0.08				
GFROUP B (PI) PRE	0.31	0.03	-0.01	0.14	0.106
POST	0.24				
GROUP B (GI) PRE	0.52	0.05	0.02	0.25	0.020*
POST	0.38				
GROUP B (OHI) PRE	0.91	0.10	0.04	0.51	0.021*
POST	0.63				

(Group A- Colgate anticavity, Group B- Purexa toothpastes, SI-Stain Index, PI-Plaque Index, GI-Gingival Index, OHI-Oral hygiene Index; PRE: pre intervention, POST: post-intervention). $p<0.05$, Statistically significant*

Table 3: Inter group comparison of means of both the bacteria pre- and post-intervention using independent t test

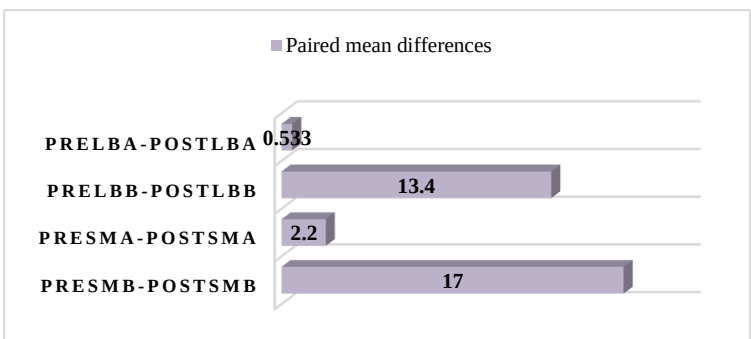
Variable	t	df	Sig.	Mean	Mean difference	Standard error difference	95% confidence interval of the difference Lower	Upper
STM	-3.151	28	0.004*	A- 2.20 B- 17.00	-14.80	4.697	-24.42	-5.17
LB	-2.96	26.95	0.006*	A- 0.53 B- 13.40	-12.86	4.34	-21.75	-3.96

STM- Comparison of means of Streptococcus mutans values between two groups, LB- Comparison of means of Lactobacilli values between two groups) $p<0.05$, Statistically significant*

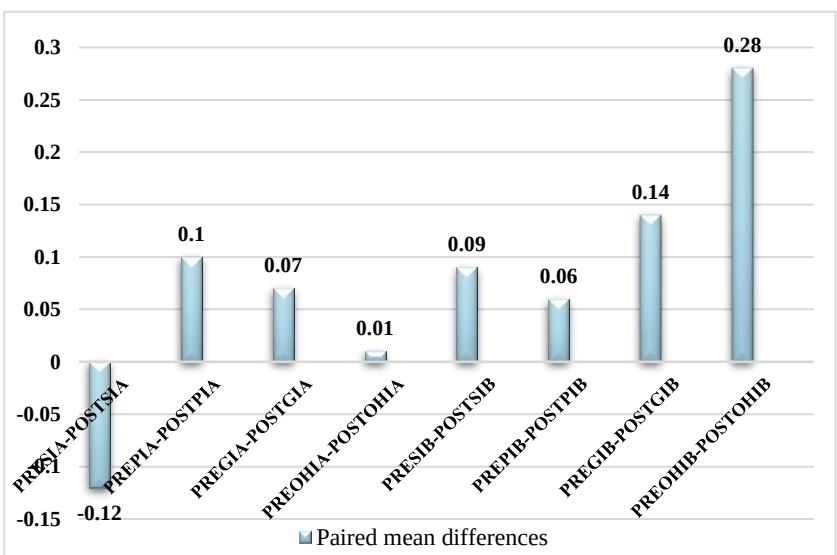
Table 4: Inter group comparison of mean differences of indices values between the 2 groups at Baseline and post intervention

Variables	t	df	Sig.	Mean difference	Standard error difference	95% confidence interval of the difference	
						Lower	Upper
Stain Index	-2.449	28	0.02*	-0.21	0.08	-0.391	-0.034
Plaque Index	0.80	28	0.42	0.04	0.05	-0.06	0.14
Gingival Index	-0.99	28	0.32	-0.06	0.06	-0.20	0.07
Oral Hygiene Index	-2.42	28	0.02*	-0.26	0.11	-0.49	-0.04

Graph 1: Intra group comparison of Colony Forming Units of both the bacteria between the 2 groups- Baseline and post intervention using paired t test



Graph 2: Intra group comparison of indices' values within the 2 groups at Baseline and post intervention



DISCUSSION

This study has been performed to determine the anticaries effect of probiotic toothpastes microbiologically, compared to conventional toothpastes. Since the prevalence of dental caries in India is higher in adults (3), the young adults age group was chosen to conduct the study with an aim to priorly introduce the probiotics oral care products among the population. There was a statistically significant difference found in both the Lactobacilli and Streptococcus mutans bacterial count in the test group between pre and post intervention. This can be due to the fact that the probiotic bacteria have the ability to competitively take down the nutrition available to pathogens and the bonding ability to the tooth surface, the pathogenic oral microbial flora might not have the capacity to fully invade the tissues of the oral cavity.

Similar to the current study it was found that there is also a significant reduction of bacterial count of Streptococcus mutans in a study (25) in 2016 through probiotic ice creams given to children for a week. In addition to that there was also a statistically significant differences of mean found in the scores after intervention in both gingival and oral hygiene indices in Group B. This may be due to the mechanism of action of the probiotics in oral cavity to compete and intervene while forming bacterial bonds and also due to the production of chemical substances like bacteriocins to inhibit oral pathogenic microorganisms' formation. In line with the current study, a study on short- term effect of probiotic Lactobacillus reuteri consumption on the salivary microbiome profile of subjects undergoing orthodontic treatment with fixed appliances within a time period of 14 days. The study concluded that probiotic intake was associated with reduced OHI and PBI (Papillary Bleeding Index) scores (50% reduction of scores, $P<0.001$). The reduced scores of the indices were in line with significantly reduced oral pathogens, like Porphyromonas pasteri, Treponema sp., Fretibacterium fastidiosum, Kingella oralis and Propionibacterium acnes (34).

A study was conducted on effect of probiotic tablets strains on gingival inflammation and composition of the salivary microbiome in a randomized placebo-controlled trial with a 4-week intervention of probiotic tablets having Lactobacillus rhamnosus PB01, DSM 14869 and Lactobacillus curvatus EB10, DSM 32307 or placebo. It was found that Plaque Index was less affected overall, though there was presence of slight tendency of lessened plaque levels in the group of probiotic at initial follow-ups at 2 and 4 weeks. This finding stands with the current study where there was no statistically significant difference between the groups Plaque Index values at baseline and post intervention (35).

The ingredients of the probiotic dentifrice used in the current study are 35 x 106 million CFU/gm helpful bacteria bacillus coagulum (0.02%), sodium monofluorophosphate (0.8%), xylitol (2%), calcium carbonate (42.4%), hydrated silica (3%), sodium citrate (0.25%), and sorbitol (28%) where bacillus coagulum is known to inhibit the growth of cavity causing S. mutans(36). Probiotic strains of B. coagulans exert various beneficial effects, such as modulation of the microbial composition, alteration of immune responses and metabolism along with the production of various antibacterial products including bacteriocins and enzymes(37). These might be the reason for the reduction of the bacterial count in the intervention group in the current study.

CONCLUSION

The use of probiotics in various forms for improving oral health and prevention of caries is turning heads. Several studies from literature conducted with probiotic strains formerly recommended for gut health were fruitful. The current study concluded that there will be a significant reduction of Streptococcus mutans and Lactobacilli counts accompanied with significant reduction of scores of Oral Hygiene, Gingival and Plaque Indices when probiotic toothpastes are used for a two-week period. In the foreseeing future, extensive studies

conducted for longer time period or synergetic effect of the probiotics on cariogenic bacteria, oral health and optimum dosage of the probiotic organisms are still need to be discovered.

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Conflicts of interest

There are no conflicts of interest

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