“COMPARATIVE EVALUATION OF EFFICACY OF GREEN TEA AND CHLORHEXIDINE MOUTHRINSE IN REDUCING THE MUTANS STREPTOCOCCI COUNT IN SALIVA - A RANDOMIZED CONTROLLED TRIAL.”

Dissertation submitted

in partial fulfillment of the requirements

for the degree of

MASTER OF DENTAL SURGERY

BRANCH – VII

PUBLIC HEALTH DENTISTRY

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600 032

2014 – 2017

DECLARATION BY THE CANDIDATE

I hereby declare that this research titled **"COMPARATIVE EVALUATION OF EFFICACY OF GREEN TEA AND CHLORHEXIDINE MOUTHRINSE IN REDUCING THE MUTANS STREPTOCOCCI COUNT IN SALIVA - A RANDOMIZED CONTROLLED TRIAL"** is a bonafide and genuine research work done by me under the guidance of **Dr. MUTHU KARUPPAIAH.R., M.D.S.,** Reader, Department of Public Health Dentistry, Best Dental Science College, Madurai.

Priscilla Joys N.

Date: 6.01.2017

Signature of the Candidate.

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THE TAMIL NADU DR. M.G.R MEDICAL UNIVERSITY

TAMIL NADU

CERTIFICATE BY THE GUIDE

This is to certify that the research titled **"COMPARATIVE EVALUATION OF EFFICACY OF GREEN TEA AND CHLORHEXIDINE MOUTHRINSE IN REDUCING THE MUTANS STREPTOCOCCI COUNT IN SALIVA - A RANDOMIZED CONTROLLED TRIAL"** is a bonafide research work done by **Dr. PRISCILLA JOYS.N** under my guidance, in partial fulfillment of the requirements for the degree of **M.D.S** in **PUBLIC HEALTH DENTISTRY** degree examination.

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ENDORSEMENT BY HEAD OF THE DEPARTMENT AND PRINCIPAL/ HEAD OF THE INSTITUTION

This is to certify that the research titled **"COMPARATIVE EVALUATION OF EFFICACY OF GREEN TEA AND CHLORHEXIDINE MOUTHRINSE IN REDUCING THE MUTANS STREPTOCOCCI COUNT IN SALIVA - A RANDOMIZED CONTROLLED TRIAL"** is a bonafide research work done by **Dr. PRISCILLA JOYS.N** under the guidance of **Dr. MUTHU KARUPPAIAH.R. M.D.S.,** in partial fulfillment of the requirements for the degree of **M.D.S** in **PUBLIC HEALTH DENTISTRY** degree examination.

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For who hath despised the day of small things?

-Zechariah 4:10

What started as a small, trivial idea has metamorphosed and taken the shape of this book. I thank ***my Almighty God,*** the Author and Finisher of my faith for bestowing His abundant blessings and grace throughout this research and my post graduation journey.

With all due reverence, I express my sincere gratitude to our Honourable Chairman ***Prof. Dr. K.R.Arumugam M.Pharm.,*** and our Respected Vice Chairman ***Prof. Dr. A. Babu Thandapani M.Pharm., Ph.D.,*** for their blessings and support. I thank my esteemed Principal ***Dr.Vijayalakshmi M.D.S.,*** for granting me the permission to conduct this research. I am also grateful to ***Dr.Prem Kumar M.D.S.,*** Vice Principal, for his encouragement and support.

Writing this piece of literature is by itself an important milestone in this course and I very humbly owe it to my beloved teachers who have helped me in this research.

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According to the saying, "SAVE THE BEST FOR THE LAST" – I wish to thank my parents, ***Dr.Nagarajan M.D.D.A.,*** Chief Anaesthetist, Karpagam Medical University, Coimbatore, and ***Dr.Kasthuri Bai M.B.B.S.,*** Senior Civil Surgeon., who have made me what I am today. I thank them from the bottom of my heart for giving me "the best" of everything. I extend my heartfelt gratitude to my parents-in-law ***Mr.Abel Raj.*** Livestock Inspector and ***Ms. V.P.Saroja M.A.,M.Ed.*** for their prayerful support. I hope that I be a blessing to them as much as they are to me.

Acknowledgement

I dedicate this humble piece of work to my **Fabulous Family** who make every single day and every single thing, special and worthwhile.

6.01.2017
Madurai
DATE & PLACE

Priscilla Joys.N
Dr.Priscilla Joys.N

DECLARATION

TITLE OF THE DISSERTATION	COMPARATIVE EVALUATION OF EFFICACY OF GREEN TEA AND CHLORHEXIDINE MOUTHRINSE IN REDUCING THE MUTANS STREPTOCOCCI COUNT IN SALIVA - A RANDOMIZED CONTROLLED TRIAL.
PLACE OF STUDY	BEST DENTAL SCIENCE COLLEGE, MADURAI – 625104.
DURATION OF THE COURSE	3 YEARS
NAME OF THE GUIDE	Dr. MUTHU KARUPPAIAH.R M.D.S.,
HEAD OF THE DEPARTMENT	Dr. BHARATH KUMAR GARLA M.D.S.,

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance/any promotion without obtaining prior permission of the Principal, Best Dental Science College, Madurai – 625104. In addition, I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in the dissertation. The author has the right to reserve for publish of work solely with the prior permission of the Principal, Best Dental Science College, Madurai – 625104.

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Signature of the candidate

TRIPARTITE AGREEMENT

This agreement herein after the "Agreement" is entered into on this day 6th Jan 2017, between the Best Dental Science College represented by its **Principal** having address at Best Dental Science College, Madurai – 625104, (hereafter referred to as, 'the College')

And

Dr. MUTHU KARUPPAIAH.R aged 37 years working as **Reader** in the Department of Public Health Dentistry at the College, having residence address at No 78, Minikiyur, Kovilpatti post, Manapparai Taluk, Trichy District – 621 305 (herein after referred to as the 'Principal Investigator')

And

Dr. PRISCILLA JOYS.N aged 33 years currently studying as **Post Graduate student** in Department of Public Health Dentistry, Best Dental College, Madurai- 625104 (herein after referred to as the 'PG/Research student and co-investigator')

Whereas the PG/Research student as part of her curriculum undertakes to research on **"COMPARATIVE EVALUATION OF EFFICACY OF GREEN TEA AND CHLORHEXIDINE MOUTHRINSE IN REDUCING THE MUTANS STREPTOCOCCI COUNT IN SALIVA - A RANDOMIZED CONTROLLED TRIAL."** for which purpose PG/Principal Investigator shall act as Principal Investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG/Research student as to the extent possible as a Co-investigator.

Whereas the parties, by this agreement have mutually agreed to the various issues including in particular the copyright and confidentiality issues that arise in this regard.

Now this agreement witnessed as follows

1. The parties agree that all the Research material and ownership therein shall become the vested right of the college, including in particular all the copyright in the literature including the study, research and all other related papers.
2. To the extent that the college has legal right to do so, shall grant to license or assign the copyright so vested with it for medical and/or commercial usage of interested persons/entities subject to a reasonable terms/conditions including royalty as deemed by the College.
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5. All expenses pertaining to the research shall be decided upon by the Principal Investigator/Co-investigator or borne solely by the PG/Research Student, (co- investigator).
6. The college shall provide all infrastructure and access facilities within and in other institutes to the extent possible. This includes patient interactions, introductory letters, recommendation letters and such other acts required in this regard.
7. The Principal Investigator shall suitably guide the Student Research right from selection of the Research Topic and Area till its completion. However the selection and conduct of research, topic and area of research by the student researcher under guidance from the

Principal Investigator shall be subject to the prior approval, recommendations and comments of the Ethical Committee of the College constituted for this purpose.

8. It is agreed that as regards other aspect not covered under this agreement, but which pertain to the research undertaken by the PG student, under guidance from the Principal Investigator, the decision of the college shall be binding and final.
9. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act 1996.

In witness whereof the parties herein above mentioned have on this day, month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

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BEST DENTAL SCIENCE COLLEGE
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College represented by its **Principal:**

Student Guide:

READER
PUBLIC HEALTH DENTISTRY
BEST DENTAL SCIENCE COLLEGE
MADURAI

PG Student: Priscilla Joys. N

Witnesses:

1.

2.

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LIST OF ABBREVIATIONS

S.NO	ABBREVIATION	MEANING
1.	a.m	ante meridiem
2.	AD	Anno Domini
3.	ADA	American Dental Association
4.	C	Catechin
5.	CHX	Chlorhexidine
6.	DMFT/DMFS	Decayed, Missing, Filled Tooth/ Decayed, Missing, Filled Surfaces
7.	EC	EpiCatechin
8.	ECg	EpiCatechin gallate
9.	EGC	EpiGallo Catechin
10.	EGCg	EpiGallo Catechin gallate
11.	mg/ml	Milligram per milliliter
12.	MIC	Minimum Inhibitory Concentration
13.	MS	Mutans Streptococci
14.	p.m.	post meridiem
15.	pH	power of hydrogen

16.	SES	Socio Economic Status
17.	SPSS	Statistical Package for Social Sciences

Abstract

ABSTRACT

INTRODUCTION: Dental caries is claimed to be an ubiquitous and almost universal bacterial infection that has been afflicting mankind since the days of civilization. Mutans Streptococci are the surrogate marker for dental caries as they are implicated to be the initiators of the disease and their quantity in the saliva is directly related to the number of surfaces colonized by them. Any intervention that can hamper their growth and survival will negatively impact the initiation and progress of caries. Various antimicrobial agents have been tried and tested against these micro-organisms.

AIM AND OBJECTIVES: To compare the effect of 0.2% Chlorhexidine mouthrinse and 2% Green tea extract on the Mutans Streptococci level in saliva and salivary pH.

MATERIALS AND METHODS: It is a randomized, parallel arm, controlled trial designed to compare the effect of 2% Green tea extract with 0.2% Chlorhexidine mouthrinse on the Mutans Streptococci count in the saliva and salivary pH.

RESULTS: There was a statistically significant reduction in the Mutans Streptococci colonies in the saliva after 0.2% Chlorhexidine mouthrinse. There was a statistically significant reduction in the Mutans Streptococci colonies in the saliva after 2% Green tea extract mouthrinse. There is a rise in the salivary pH towards neutrality after rinsing with the 0.2% Chlorhexidine mouthrinse but this is not statistically significant. The inhibition of the fall in the salivary pH after 2% Green tea extract mouthrinse is clearly demonstrated and is statistically significant. Significant difference was observed between the Mutans Streptococcus Count (CFU/ml) before and after rinsing with 0.2% Chlorhexidine and 2% Green tea.

CONCLUSION: 0.2% Chlorhexidine mouthrinse is superior to 2% Green tea mouthrinse in the reduction of Mutans Streptococci in the saliva. However Green tea showed promising results with respect to the inhibition of the fall in the salivary pH after the sucrose challenge.

DENTAL PUBLIC HEALTH SIGNIFICANCE: Although the antibacterial efficacy of 2% Green tea is not as effective as the gold standard Chlorhexidine, it is devoid of adverse effects like staining and taste alteration. As Green tea showed an extraordinary acid inhibition property of preventing the rise in salivary pH after a sucrose challenge, it can be considered as a promising cost-effective alternate as a mouthrinse for long term use.

KEY WORDS:

Green tea, Mutans Streptococci, Chlorhexidine, Dental Caries.

Introduction

INTRODUCTION

Dental caries is claimed to be one of the most common bacterial infections that has been afflicting mankind. However, this startling significance is overpowered by the fact that this disease is ubiquitous and it is non-life threatening. The war against dental caries had always been in the "symptomatic treatment" realm which caused an enormous economic burden to the society. Little was known about the prevention aspects of the disease. Later, with the advent of researches in the identification of the specific causative micro-organisms, Mutans Streptococci were incriminated as the initiators of dental caries.[1] Since then, reducing Streptococci load in the oral cavity is recognised as a practical step to lower the incidence of dental caries. Researches done in this direction have given us many plaque control agents.

Chlorhexidine is the gold standard chemical plaque control agent which has a broad antibacterial activity, very low toxicity, and more substantivity. Chorhexidine is a Bis-biguanide that significantly reduces the total number of bacteria in the oral cavity and also interferes with the adherence factors of these cariogenic micro-organisms on the tooth surface.[2] However, Chlorhexidine is burdened with some side effects like staining of tooth, alterations in taste and mucosal erosions besides its beneficial antiplaque effects. The search for alternative agents has brought herbal products to this field. Herbal products are being used in India since ancient times for the treatment of various ailments. The commercial use of these products in toothpaste and for oral irrigation delivery has increased manifold nowadays.[3]

Green tea is one of the herbal products which is being actively researched for its health benefits. Green tea is made solely with the leaves of *Camellia sinensis* that have undergone minimal oxidation during processing.[4] Green tea is prepared from the unfermented leaves of the plant and therefore has the maximum amount of the most active ingredients which are the polyphenols than the partially fermented and fully fermented counterparts namely the Oolong tea and Black tea respectively.[5]

Green tea has its origin in China dating back to the third century AD. It was first consumed for its medicinal properties against anxiety, depression and gastro-intestinal ailments. Gradually it rose in popularity and became a common beverage in China. Green tea is claimed to be the second most popular drink in the world, next only to water - "the elixir of life", with Japan and China being its leading producers.[6]

Green tea has been used as a traditional medicine and several studies have shown that Green tea contains a myriad of valuable compounds of which polyphenols have a number of beneficial effects on health. These include anticariogenic effects, anti-oxidative properties, reducing the risk of cardiovascular disease, anticarcinogenic effects, and powerful antimicrobial and antiviral properties. Polyphenols inhibit the growth of oral and periodontal pathogenic bacteria and can improve oral and gingival health.[7] Green tea is also considered to be an effective method to deliver fluoride into the oral cavity as it is also a natural source of fluoride. The polyphenols target the virulence factors of the Mutans Streptococci and also inhibit the adherence of micro-organisms.[8] They also inhibit acid production by the bacteria thereby increasing the pH of the saliva.[9]

In 1989, it was demonstrated that Japanese Green tea has an inhibition effect on Streptococcus mutans. *In vitro* tests reveal that the Minimum Inhibitory Concentration (MIC) of Green tea polyphenols to be 250-1000 microgram/ml.[10]

Epidemiological studies and laboratory studies have been conducted in the recent past to reveal the health benefits of Green tea. Available literature shows that there are few studies comparing the efficacy of Chlorhexidine with Green tea as an antimicrobial agent.[1] Hence an attempt has been made in this less travelled path. The aim of this research is to compare the effect of 0.2% Chlorhexidine mouthwash and 2% Green tea extract on the salivary pH and Mutans Streptococci levels in the saliva.

Aim and Objectives

AIM AND OBJECTIVES

AIM :

To compare the effect of 0.2% Chlorhexidine mouthrinse and 2% Green tea extract on the Mutans Streptococci level in saliva and salivary pH.

OBJECTIVES:

1. To assess the Mutans Streptococci level in saliva and salivary pH in the subjects, before and after 2% Green tea extract.
2. To assess the Mutans Streptococci level in saliva and salivary pH in the subjects, before and after 0.2% Chlorhexidine mouthrinse.
3. To compare the effect of 0.2% Chlorhexidine mouthrinse and 2% Green tea extract on Mutans Streptococci level in saliva and salivary pH.

Review of Literature

REVIEW OF LITERATURE

In 1989, Sakanaka et al[10] conducted an *in vitro* study to isolate the active ingredients from Green tea and also observe their mechanism of action for inhibiting bacterial growth. The active ingredients were revealed to be polyphenols like catechin, EpiCatechin, GalloCatechin (GC), EpiCatechin gallate, EpigalloCatechin (EGC) and EpiGalloCatechin gallate (EGCg). The MIC of the components GC, EGC, EGCg, is 250 micrograms/ml, 250 or 500 micrograms/ml, 500 - 1000 micrograms/ml respectively. The minimum time required for the bactericidal action of EGCg was thirty minutes, when the CFU/ml of Streptococcus mutans bacteria was reduced to one tenth of the initial value. In eight hours, the CFU/ml was reduced to almost a negligible value. The authors recommend that drinking one cup of green tea a day, which is 100 ml, contains more polyphenols that were used in this experiment and can definitely aid in the reduction of the incidence of Dental caries.

In 1991, Otake et al[8] conducted a Laboratory study on rats and an *in vitro* study to evaluate the anticariogenic effect of Green tea polyphenols. The polyphenols inhibited the adherence of Streptococcus mutans on saliva coated hydroxyapatite discs. It also inhibited the production of glucans by the bacteria. In this research, it was found out that the two polyohenols, namely, Epicatechin gallate and Epigallocatechin gallate were superior in inhibiting the glucosyltransferase activity which is responsible for the formation of glucans from sucrose. This further reduces the adsorption of the bacteria to the tooth surfaces thereby preventing their colonization in the oral cavity. This is subsequently followed by a lower incidence of caries development. The mechanism of action proposed for the decreased bacterial adhesion is also the competitive binding of the tannins to proline rich proteins in the saliva and the bacterial cell surfaces, thereby making them unavailable for adhesion.

The experiment also included eight groups of rats with seven rats in each group. The groups differed in terms of the diet fed to the rats. Some groups were fed with food and/or water containing polyphenols from Green tea. Rats which were fed with water containing polyphenols had lower caries score compared with the rats in the control group.

In 2006, Hirasawa et al[11] conducted an *in vitro* study to assess the activity of an ingredient of Green tea which is Epigallocatechin gallate (EGCg) at a concentration of 2mg/ml on dental plaque pH after rinsing with 10% sucrose. There was a statistically significant higher plaque pH values after treatment with the catechin. This shows that Green tea catechins inhibited acid production from plaque. The effect of the catechin on the survival of Streptococcus mutans and Streptococcus sobrinus was also analysed in both sucrose containing medium and in a medium without sucrose. Bacteria in the former culture medium were unaffected whereas those in the latter medium were killed. The authors discuss that inhibition of acid production from the cariogenic bacteria which are usually glucan coated in a strategy for bactericidal activity of the EGCg. It is also found out that sufficient time is required to allow for the catechin to penetrate into the dental plaque. Catechins like EGCg and ECg which have the galloyl radical inhibit lactohydrogenase activity. A concentration of more than 2mg/ml of EGCg is advocated for the effective use of the catechin as a mouthrinse.

In 2006, Molinari M et al[12] in their case report, describe a patient who was admitted in the Intensive care unit of a hospital in Halifax, Canada, with symptoms of malaise and abdominal pain. After a detailed history taking, clinical examination, and laboratory investigation, it was suggested that this person was taking dietary supplements of Green tea extract in a dosage of 720 mg/day for the last 6 months

prescribed for weight loss. The authors are yet unclear of the mechanism of action of the Green tea extract causing the adverse effect on liver. The extent of damage was so severe that the patient required liver transplant. The authors also discuss series of cases of acute hepatitis reported elsewhere, all caused possibly due to use of Green tea extracts as dietary supplements. The authors urge the necessity to take a clear and detailed history of all dietary supplements when a patient reports with symptoms of acute hepatitis. The authors stress that many herbal products which enter the market with the aura of "being natural" come without sufficient clinical trials to support their efficacy in human beings and safety for consumption.

In 2010, Chacko et al[13] in their review have described the various beneficial effects of Green tea on health and the possible harmful effects of the same. The literature is a review of a hundred and five peer-reviewed papers in English. The beneficial effects of Green tea on health like, anti-tumorogenic property, immune modulations, antioxidant property, hypolipidemic property, antimutagenic and anti carcinogenic property, antifungal property, antibacterial effects and neuroprotective properties which have been substantiated by lab and animal studies have been outlined. The main active ingredients of the Green tea are the polyphenols also called as catechins which include Epicatechin, Epigallocatechin, Epicatechin 3 gallate, Epigallocatechin 3 gallate. They constitute 30% of the dry weight of the green tea leaves. Epigallocatechin 3 gallate is the most active and majority of the health benefits are attributed to it. These catechins reduce serum glucose levels and plasma triglycerides. They help fight obesity by raising the postprandial thermogenesis and oxidation of fat. Adverse effects of green tea like hepatotoxicity, DNA damage of pancreas and liver, thyroid enlargement, are also noted. These effects are owed to the

aluminium content, the caffeine content and the effect of Green tea on iron bioavailability.

In 2011, Naderi.J et al[7] conducted an *in vitro* study to determine the efficacy of organic and aqueous extracts of varying concentrations of Green tea and Black tea against Streptococcus mutans activity. The mean zones of inhibition were calculated. The Minimum inhibitory concentration and the minimum bactericidal concentrations were also calculated The MIC of the methanolic extract of Green and Black tea were 150 mg/ml and 50 mg/ml respectively. Lower concentration of Black tea compared to green tea was required to show antibacterial activity. Catechin content of the tea leaves vary with respect to geographical region, soil, climate and the type of processing. The mean diameter of inhibition zone was 9.5 mm for Green tea and 10.9 mm for Black tea.

In 2011, Gianmaria et al[14] conducted a study to assess the efficacy of 40mg/ml Green tea extract against Mutans Streptococci and lactobacilli in the saliva of 12 to 18 year old subjects. A total of 66 patients were enrolled in the study and equally divided into 2 groups of 33 participants in each group. The control group were given a placebo. Saliva samples were taken at 4 days and 7 days after the baseline. The colonies were counted using chair-side kits. There was a significant statistical difference between the experimental and control groups, both in terms Of Mutans Streptococci and Lactobacilli. Green tea was suggested to be a promising natural anticariogenic agent.

In 2011, Awadalla et al[9] conducted a study to assess the efficacy of Green tea in reducing Streptococcus mutans levels in the plaque and saliva and its effect on the salivary and plaque pH and the influence on gingival health. It was a pilot study conducted on 25 patients (13 males and 12 females) who were within the age group of 21 to 46 years. A 10% sucrose challenge was given to the patient during the research. 2% Green tea was given to be rinsed for 5 minutes There was a significant statistical difference in the Streptococcus mutans count in the saliva and plaque as well as the pH values and the gingival bleeding Index, pre and post-rinse with a 2% Green tea extract. The results suggested Green tea to be a possible caries preventive agent especially in developing countries.

In 2012, Subramanium P et al[15] conducted an in vitro study to determine the effect of aqueous, ethanolic and methanolic extract of the three types of tea, namely Green tea, Oolong tea and Black tea. This was compared with 0.2% Chlorhexidine. The Zones of inhibition were compared. Green tea exhibited a greater zone of inhibition compared to Chlorhexidine. Although all extracts showed inhibitory effect on the bacteria, aqueous extract of oolong tea was superior in the inhibition which was displayed by the greatest zone of inhibition. Catechins are the bioactive molecules in tea leaves. They act by antimicrobial mode of action and not by influencing demineralization and remineralization. EGCg and EC disrupt bacterial membranes. Tea extracts on the whole can be used as effective caries preventive agents and incorporated into dentifrices, mouthrinses, chewing gums and dental floss.

In 2013, Moeezizadeh M[16] in the review has listed out the possible mechanisms of Green tea against dental caries. Tea is one of the popular beverages consumed by people in the world. There are three types of tea depending upon the level of processing the leaves of the plant Camellia sinensis. The dried and steamed

processing inactivates enzymes that deteriorate the green colour of the leaves, thereby helping to retain their natural color, and so called Green tea. They are rich in polyphenols. The semi-fermented version is the oolong tea which contains considerable catechins. The third variety is the completely fermented tea leaves called as black tea. It contains theaflavins and thearubigins. The caries inhibitory effects of tea is because of the direct bactericidal effect of the polyphenols. They also inhibit plaque formation by interfering with the adherence of bacteria on the tooth by inhibiting the glucosyl transferase activity that converts sucrose to glucans. The fluoride content also exerts a considerable cariostatic action. This fluoride is believed to inhibit the demineralization of dentin in the caries process. The constituents in black tea also are found to attenuate the progress of dental caries, even in the presences of dietary sugars. However, the exact mechanism of caries inhibition is yet to be explored by further studies.

In 2014, Neturi et al[17] conducted a study to compare the antibacterial efficacy of 0.2% Chlorhexidine, and 2% Green tea on the plaque samples. It was a cross over trial with a washout period of 7 days. It was conducted on 30 subjects who were 20-25 years old belonging to both genders. Ten ml of each intervention was given to be rinsed for one minute. Following this, after five minutes, plaque samples were collected and cultured in chocolate agar. The results showed that both the mouthrinses reduced Streptococcus mutans colonies significantly. The reduction of Streptococcus mutans colonies by Chlohexidine group was slightly more than the reduction in the Green tea group. Hence, Green tea was suggested to be comparable with Chlorhexidine in reducing Streptococcus mutans colonies and thereby recommends it to be an economical public health intervention.

In 2014, Rao.A et al[18] conducted an *in vitro* study to compare the antibacterial efficacy of 2% Green tea and 0.12% Chlorhexidine on Streptococcus mutans in saliva samples. A total of 30 salivary samples were used in the study. Three groups with 10 samples each were formed. The positive control was 0.12% Chlorhexidine and the negative control was normal saline. The Green tea used here was a 2% hydroalcoholic extract prepared from pulverized green tea leaves. One ml of the saliva samples were added to 1 ml of the interventions and were transferred to trypticase soy agar culture media. The colonies were counted after 24 hours of incubation at 37^0 C by an independent interpreter. Both Green tea and Chlorhexidine showed significant statistical reduction of Streptococcus mutans colonies. However, Chlorhexidine was superior to green tea in its antibacterial efficacy. Green tea is suggested to be a promising alternate to Chlorhexidine in patients as it is devoid of adverse side effects like dental staining and genotoxic effects that accompany the longterm use of the latter mouthrinse.

In 2014, Kaur et al[4] conducted a randomized controlled trial to compare the antiplaque effectiveness of Chlorhexidine and Green tea mouthwash. It was a crossover trial conducted on 18 – 25 year old patients, in two phases on one week each. A fifteen day washout period was given. A total of 30 participants were included in the study, with fifteen in each group. The interventions used were 0.12% Chlorhexidine and 0.25% green tea catechin mouthwash. The mouthrinses were used twice daily by the participants in a dose of 15ml for one minute. Turesky modification of Quigley-Hein plaque index was used to evaluate the effect at the end of each phase by a single examiner. Plaque scores were recorded after using disclosing agent. Plaque scores on buccal surfaces, anterior teeth, lingual surfaces and whole dentition were scored. The mean plaque score for Green tea was 2.8333 ± 0.32940 and for

Chlorhexidine, it was 2.8467±0.32940. No significant difference was found in the plaque scores between the two groups ($p>0.05$). This result was attributed to the antibacterial effects on the plaque forming micro-organisms, exerted by the Green tea catechins. Both Green tea and Chlorhexidine showed comparable results. Green tea was suggested to be a long-term and cost-effective mouthrinse.

In 2014, Fajriani et al[19] conducted a study to compare the efficacy of 2.5% Green tea and 0.2% Chlorhexidine in reducing Streptococcus mutans levels in saliva in children aged 6 – 12 years. Saliva samples were collected at baseline and then at 15th minute and 30 minutes post the interventions. Streptococcus mutans colonies were compared before and after the interventions as well as between the groups. There was a significant reduction between the Streptococcus mutans colonies before and after the mouthrinses at 15 minutes in both the groups. However the statistical difference between the groups was not significant.

In 2015, Thomas.A et al[20] conducted a study to compare the effect of Chlorhexidine (0.2%), Alum (0.02 M), Fluoride with essential oils (0.05%), Sodium fluoride (0.05%), 0.5% Green tea, and Garlic with lime mouth rinses against Lactobacillus, Candida albicans, and Streptococcus mutans. Chlorhexidine mouthrinse was the most effective against Streptococcus mutans. The mean zones of inhibition was 18.667 and 10.833 for Chlorhexidine and Green tea respectively. Garlic with lime was found to be the most effective natural mouthrinse. Green tea was shown to have a modest effect on Lactobacillus and Candida albicans count when compared with the other mouthrinses.

In 2016, Cardoso, KB et al[21] conducted a study to compare the efficacy of Green tea mouthrinse with a placebo, namely 0.9% saline. The trial was conducted on eighty patients. The participants belonged to 18 – 61 years old age group, who suffered from gingivitis. They were instructed to use 10ml of 20mg/ml of Green tea mouthrinse for thirty seconds, twice daily for a period of 15 days. Oral soft tissues were clinically examined. Plaque index and gingival index (Loe, 1967) were scored and compared. The statistical differences obtained in the gingival index scores were not significant. However, the statistical differences obtained in the plaque index scores were significant. The influence of the Green tea active ingredients on the plaque formation mechanism might be the possible reason behind the reduction of plaque scores. The use of Green tea mouthrinse for a period of 15 days did not cause any adverse side effects in the oral soft tissues. The authors conclude by advocating further studies on long term basis to elucidate the effects of green tea on oral soft and hard tissues.

Materials and Methods

MATERIALS AND METHODS

STUDY DESIGN:

The present research is a randomized, parallel arm, controlled trial designed to compare the effect of 2% Green tea extract with 0.2% Chlorhexidine mouthrinse on the Mutans Streptococci count in the saliva and salivary pH.

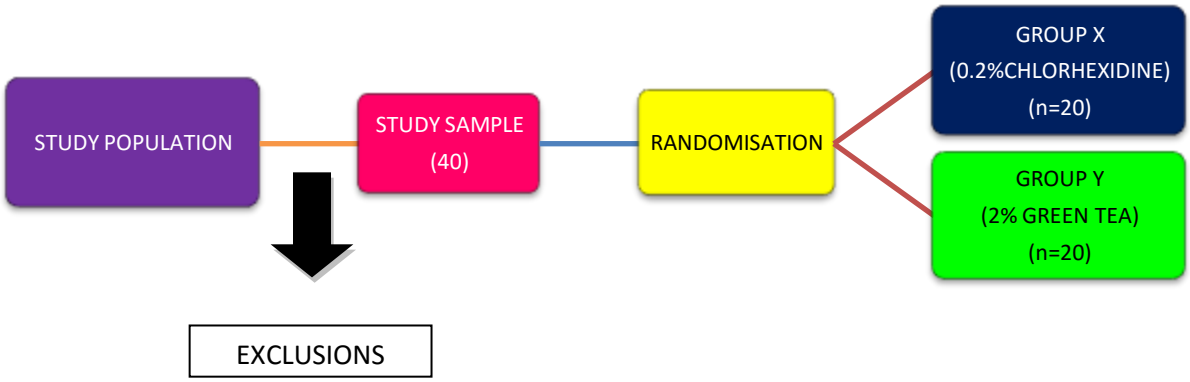


Figure 1: Study design - parallel arm, Randomised controlled trial with two groups

STUDY AREA

Madurai is an important city, located in the south of the state of Tamil Nadu in India. It is the administrative headquarters of Madurai District and the 31st largest city in India. Madurai is the third largest city by population in Tamil Nadu. The history of Madurai is intertwined with the history of the Tamil Language and the third Tamil Sangam, a chief assembly of Tamil scholars, is believed to have been conducted in the city.

EDUCATIONAL INSTITUTIONS IN MADURAI

Madurai has several educational institutes. Madurai is said to be the cultural hub of Tamil Nadu. Madurai is considered as an important centre of learning for Tamil culture, literature, art, music and dance. Madurai Kamaraj University, is a state-run university which has 109 affiliated arts and science colleges in Madurai and neighbouring districts. There are 47 approved institutions of the university in and around the city.[22] The government website for Madurai city lists out 70 different colleges in and around Madurai. They include, Arts and science colleges, Management colleges, Music colleges, Preceptors colleges, Law college, Medical and Engineering colleges, Paramedical institutes, Polytechnique and I.T.I colleges.

ETHICAL CLEARANCE:

The nature and purpose of the study was explained to the Institutional Review Board and ethical clearance was obtained. (Annexure -I) and permission to conduct the pilot study and the main study in the colleges was granted by our institution. After explaining the research in detail, in simple and comprehensible language with the help of the patient information letter, Informed consent was obtained from the research participants (Annexure -II)

PERMISSION BY THE COLLEGE AUTHORITIES:

The head of the institutions of the college were approached by the principal investigator. The aim and objectives of the research and the methodology was explained and permission was sought to conduct the research among the students. In the class rooms, the aim and objectives of the research was explained to the students. Only those who consented to participate in the study were included.

SOURCE OF DATA:

The source of data was primary in nature which included clinical examination and stimulated saliva collection for microbiological analyses.

STUDY POPULATION:

The study population includes only those college going students who were 17 – 25 years old, attending educational institutions in Madurai.

ELIGIBILITY CRITERIA

INCLUSION CRITERIA:

1. Caries active individuals in the age group of 17 – 25 years old.

   Caries active individuals are those who have at least one carious tooth surface.[1]
2. Those who brush only once daily

EXCLUSION CRITERIA:

1. Students not willing to participate.
2. Students who received antibiotic therapy for the last 2 weeks
3. Students who had topical fluoride application for the last 48 hours
4. Students who had topical mouth wash application for the last 48 hours and
5. Students who ate or drank within last 1 hour before the study
6. Students with limited manual dexterity.
7. Students with systemic illness/mental illness/physically handicapped
8. Students giving history of allergy to any of the above constituents of mouthrinses.
9. Pregnant and lactating women.

SAMPLE SIZE DETERMINATION:

The sample size for the present study was determined scientifically. The data required for determining the sample size was imported from a previously published scientific article. The required sample size in each group was calculated using the following formula.

$$n = \frac{2 \times \{z_{(1-\alpha/2)} + z_{(1-\beta)}\}^2}{\Delta^2}$$

Where $z_{(1-\alpha/2)}$ is the alpha error whose value for significance level of 0.5% (confidence level of 95%), is 1.96 and $z_{(1-\beta)}$ is the beta error or power of the study whose value power of 90% is 1.2816 and

$$\Delta^2 = \frac{(p_1-p_2)^2}{p'(1-p')} \quad \text{where } p' = \frac{p_1+p_2}{2}$$

where p_1 and p_2 are the percentage of reduction in mutans streptococci level in saliva in the two groups.

In similar studies conducted by Kulkarni et al[23] and H.I.Awadalla et al[9] the authors had reported 93.29% (p_1 = 0.9129) and 41.86% (p_2= 0.4186) as percentage of reduction in mutans streptococci level in saliva.

$$\Delta^2 = \frac{(0.9329-0.4186)^2}{0.6758(1-0.6758)}$$

$$= 1.2072$$

Alpha error at 0.5% significance level = 1.96

Beta error (power) at 90 % = 1.2816

Sample size n $= \frac{2\,(1.96 + 1.2816)^2}{1.2816} = \frac{2(3.2416^{2})}{1.2816}$

$= \frac{21.01594}{1.2816}$

= 17.41 rounded off to 18

Making an allowance of 10% for attrition, the required sample size for each group is calculated to be 20.

Total sample size for the two groups = 2 x 20 = 40

The required sample size is 40 cases for a significance level of 0.5 % (confidence level of 95%) and power of 90%.

EXAMINER CALIBRATION:

Training exercises were first carried out in the Department of Public Health Dentistry, Ultra's Best Dental Science College and Hospital on the out-patients under the guidance of a trained person. About eight subjects were examined to assess the consistency of intra-examiner reproducibility. The agreement for most assessments was expected to be 90%.

PILOT STUDY:

Pilot study was conducted in Thiagarajar college of Preceptors in the month of May 2016. (Annexure –III). 28 subjects, 14 in each group were recruited and pilot study was conducted to check the feasibility of the research. Practical issues like the acceptability of the mouthrinses by the subjects, saliva collection, the time taken to transport the samples to the lab, microbiological analyses of the samples, testing of pH of the saliva, were all tested during this pilot study. Appropriate adjustments were made in the methodology and implemented in the main study.

SAMPLING FRAME

As mentioned above, the government website for Madurai city lists out 70 different colleges in and around Madurai. Lottery method was used to narrow down to Thiagarajar college of preceptors and Senthamil College, Madurai from this list. Pilot study was conducted in the former college and the main study was conducted among the students in the latter college. (Annexure IV).

The subjects who fulfilled the above mentioned criteria were recruited from a pool of students studying in this college, for the experiment group and control group.

SAMPLING METHODOLOGY

After obtaining permission from the Principal of the college, a screening camp was conducted in the college premises to screen all the students in the college. A list was prepared after the initial screening examination of all students in the college. The principal investigator maintained this "master list" of all those students in the college who were eligible to participate in the study based on the inclusion and exclusion criteria. Using "simple random sampling" students were randomly picked from this list to participate in the study based on their availability.

BLINDING

It is a double blinded study. The Principal investigator was blinded to the interventions. The Guide of the Principal investigator assigned the codes "X" and "Y" to the interventions and this was disclosed to the Dean of the Ultra's College of Pharmacy through a letter. (Annexure -V) The code was revealed to a staff in the Pharmacy college, who prepared the green tea extract and dispensed the two mouthrinses into the respective containers every day before handing them over to the

principal investigator. The interventions were kept in identical opaque containers. They were each labelled as either code "X" or "Y" in differently coloured labels. Each group was given a specific colour for easy identification. Orange for code "X" and Yellow for code "Y". The Participants were also blinded. The codes were broken only after the clinical trial was completed.

RANDOMIZATION:

Randomization was done to minimize "allocation bias". A box containing concealed envelopes was used for this purpose. Each envelope carried either code "X" or code "Y". The enrollment was stratified by gender to ensure that equal number of males and females were allocated to each group (n=10 males and 10 females in each group) Participants who were selected to participate in the study were each asked to pick a concealed envelope from the box. Participants were allocated to the respective group. Participants were given individual identity cards carrying a Unique "Participant Identification Number".

COLLECTION OF DATA:

MATERIALS USED IN THE STUDY:

1. Diagnostic instruments: mouth mirrors , tweezers and No:23 Explorers
2. Sterile Gloves and Mouth masks.
3. Salivary pH strips (Range 6.5 – 9)
4. Sterilized rubber bands.[24]
5. Sterile containers for collection of salivary samples.
6. Cold storage box with ice packs.

7. Stopwatch.
8. Data recording forms.
9. 2% Green tea extract
10. 0.2% Chlorhexidine gluconate
11. Culture media for mutans streptococci. M259-Mitis Salivarius Agar with 1% potassium tellurite (HiMedia, Batch number: 228506) CMS208 Bacitracin (HiMedia, Batch number: 249178).

INTERVENTIONS:

PREPARATION OF 2% GREEN TEA EXTRACT and 10% SUCROSE CHALLENGE:

2% Green tea was freshly prepared everyday at the Ultra's College of Pharmacy. The method is described below.

Packaged Green tea leaves were procured from a local departmental store at Madurai. (Kannan Green tea, Batch number: 231014, Manufactured and packed by Shri Kannan Departmental Store (P) Limited, Neelambur, Coimbatore.

Two grams of green tea leaves were measured in an electronic weighing scale (Electronic Balance, BL-220H, No:D432902897, Shimadzu Corporation). 100 ml of distilled water was measured in a measuring cylinder and was heated in the heating mantle (Guna Heating Mantle, Sl.No:1045, Guna enterprises, Chennai 600015) to a temperature of 60^0C. The container was removed from the heating mantle. Two grams of Green tea leaves were added to this water and set aside for ten minutes. After ten minutes, this mixture was filtered using a filter paper and funnel. 10 ml of this

2% Green tea extract was measured using a measuring cylinder and was dispensed into individual containers ready to be dispatched for use by the study subjects. 2% Green tea extract was freshly prepared everyday in this same method.

A 10% sucrose challenge was also prepared with the help of the pharmacy college. 10 grams of sucrose powder was mixed with 100 ml of purified drinking water to make the 10% sucrose challenge.

0.2% CHLORHEXIDINE GLUCONATE MOUTHRINSE:

Commercially available 0.2% Chlorhexidine gluconate mouthwash (Rexidine, Batch number:RAQ7A63, Warren, Indoco) was used as positive control. Chlorhexidine was chosen as positive control as it is hailed as the "gold standard" mouthwash.[25] Chlorhexidine exhibits both bactericidal and bacteriostatic effects depending on the concentration. However, the property of substantivity is a unique characteristic of this chemical plaque control agent.[25] Previous researches have documented the initial suppression of Mutans Streptococci in the saliva after administration of chlorhexidine.[26]

INFECTION CONTROL:

Sufficient numbers of instruments were carried to the site of the trial to avoid any interruption in the examinations. The used ones were washed and drained well before sterilization. Proper use of instruments was ensured to obviate digital manipulation in order to minimize the risk of cross-infection. Disposable gloves and masks were used. The wastes generated during the examination and the trial was disposed appropriately.

1. PREPARATION OF SPECIAL PROFORMA

A special proforma was prepared to collect the required data. (Annexure -VI) The proforma was prepared in English language. The proforma was divided into three different sections.

- The first section contained provision to record patient's demographic details such as Name, Age/Gender, Address, Phone Number And Socioeconomic Status (according to Kuppuswamy's Classification, 2013 Modification).[27]
- The second section contained provision to record patient's past medical and dental history, oral hygiene practices and other habits.
- The third section contained tables to record Decayed, Missing and Filled teeth index and Decayed, Missing, And Filled surfaces Index - (Henry.T.Klein, Carrole.E.Palmer And Knutson. J.W , 1938 – Original Index)[28]

A separate provision for recording informed consent was prepared and consent was taken from the subjects before the start of the study.

2. CLINICAL EXAMINATION:

A single examiner, the investigator, carried out the clinical examination of all the study subjects involved in the study under artificial light using standardized instruments ADA specification type III examination was followed. Participants were seated comfortably on an ordinary chair and examined. Oral examinations were conducted using a plain mouth mirror and an explorer. Only 10 patients per day were recruited in the research.

Dental caries experience was assessed using appropriate armamentarium and Decayed, Missing and Filled teeth index and Decayed, Missing, And Filled surfaces

Original Index - (Henry.T.Klein, Carrole.E.Palmer and Knutson. J.W, 1938) were scored.

The criteria for identification of Dental caries according to this index is:

1) The lesion is clinically visible and obvious,
2) The explorer tip can penetrate deep into soft yielding material,
3) There is discoloration or loss of translucency typical of undermined or demineralised enamel.
4) The explorer tip in a pit or fissure catches or resists removal after moderate to firm pressure on insertion and when there is softness at the base of the area.

3. SALIVA COLLECTION:

The purpose and method of saliva collection was explained to the research participants. All saliva samples were collected between 9.30 a.m to 12 p.m from the research participants who were clearly instructed to not to consume any food or drinks except water for at least one hour before the sample collection. The participants were asked to sit relaxed in normal chairs and benches. The Stimulated whole saliva was collected by "spitting" method.[29] Saliva was stimulated using sterilized rubber bands. A presampling time of 1 minute was given for the participant to chew the bands. The participants were instructed to chew the bands and spit the saliva into the sterile labelled containers for the next five minutes. The minutes were timed with a stopwatch. Saliva samples containing visible blood were discarded.

Four saliva samples were collected. A pre rinse stimulated salivary sample (sample A) was obtained from the subjects 1 hour after breakfast, after which a 10% sucrose solution was given to be rinsed for 2 minutes. After 7 minutes, Post rinse samples

(sample B) were collected in the same manner. Subjects were made to rinse the oral cavity with water and after a waiting period of one hour, a pre rinse saliva sample (sample C) was collected. Following this, 2% Green tea or 0.2% Chlorhexidine rinse was given for the respective groups. After a 20 minute interval, a 10% sucrose solution was given to be rinsed for 2 minutes. After 7 minutes, Post rinse samples (sample B) were collected in the same manner. All salivary samples were stimulated using sterilized rubber bands. The pH and Mutans Streptococci count of all the samples were estimated. The sterile containers were quickly closed in order to avoid contamination and store in cold storage box and were transported to the Microbiological lab within 2 hours.

DETERMINATION OF pH OF SALIVA:

Salivary pH was determined with the help of pH strips that measured the pH of the saliva by colour change. The changed colour of the pH strip was matched against the colour index that accompanied the strips and the corresponding values were recorded. It ranged from 6.5 to 9.

MICROBIOLOGICAL ANALYSIS - ENUMERATION OF MUTANS STREPTOCOCCI:

M259-Mitis Salivarius Agar with 1% potassium tellurite (HiMedia, Batch number: 228506) CMS208 Bacitracin (HiMedia, Batch number: 249178) were used to culture the Mutans Streptococci. This culture medium is regarded as a gold standard for the isolation of Streptococcus mutans, Streptococcus rattus and Streptococcus sobrinus.[1] The agar was prepared according to the manufacturer's instructions. The stimulated saliva was collected in a sterile container for the enumeration of Mutans Streptococci. The collected saliva samples were transported to the microbiology lab using a cold

storage box with frozen ice packs. The agar plates streaked with the collected saliva sample was incubated anaerobically in an incubator at 37 ^{0}C for 48 hours. After 48 hours the colonies were identified with their appearance of short, purple chains in the agar. They were gram positive and Catalase negative.[30] They were counted by the lab technician and it was recorded as CFU/ml.

EMERGENCY CARE AND REFERRAL:

In case of any emergencies that requires immediate attention, the participants were provided with the phone numbers of the principal investigator and the guide. Address of the Dental college and Hospital was also provided for referral care.

HEALTH EDUCATION:

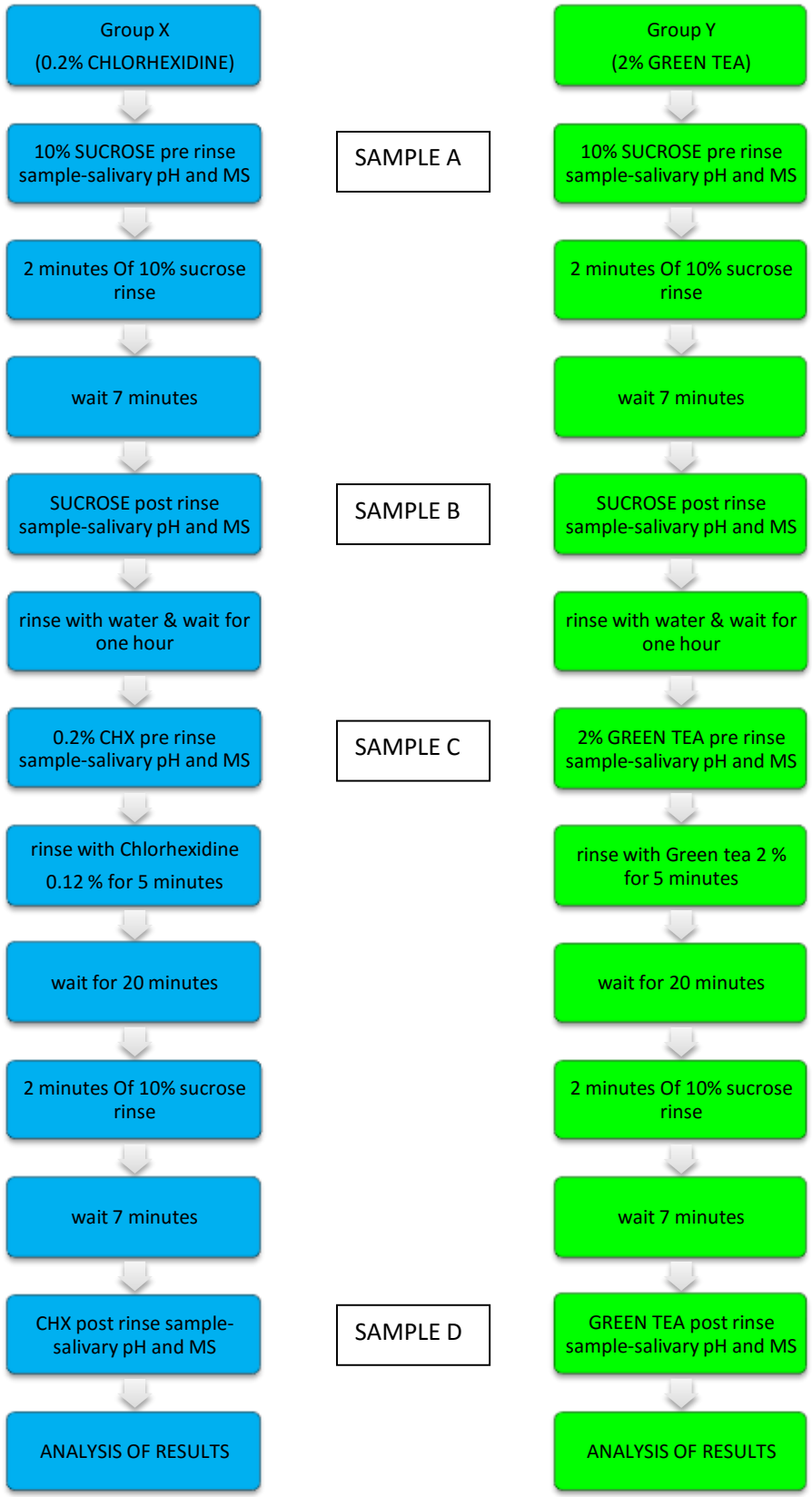
The participants were given health education that contained information on the pathophysiology of dental caries and gingivitis. Modalities of preventing them were also discussed with the help of flipcharts and slide presentations. Oral hygiene instructions were given and a oral hygiene kit containing a toothbrush and toothpaste along with a Oral hygiene instruction pamphlet was distributed.

STATISTICAL ANALYSIS:

The information collected regarding all the selected cases were recorded in a Master Chart. Data analysis was done with the help of computer using **Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, version 22 for Windows).** Using this software range, frequencies, percentages, means, standard deviations, chi square , 't' value and 'p' values and correlation coefficients (Pearson's and Spearmans) were calculated. Paired and unpaired 't' tests were used to test the significance of difference between quantitative variables and Yate's and Fisher's chi square tests for

qualitative variables. A 'p' value less than 0.05 is taken to denote significant relationship. If the absolute value (±) of correlation coefficient is more than 0.5, then there exists correlation between the two variables. Microsoft PowerPoint was used to prepare the graphs.

Flowchart of Study Procedure



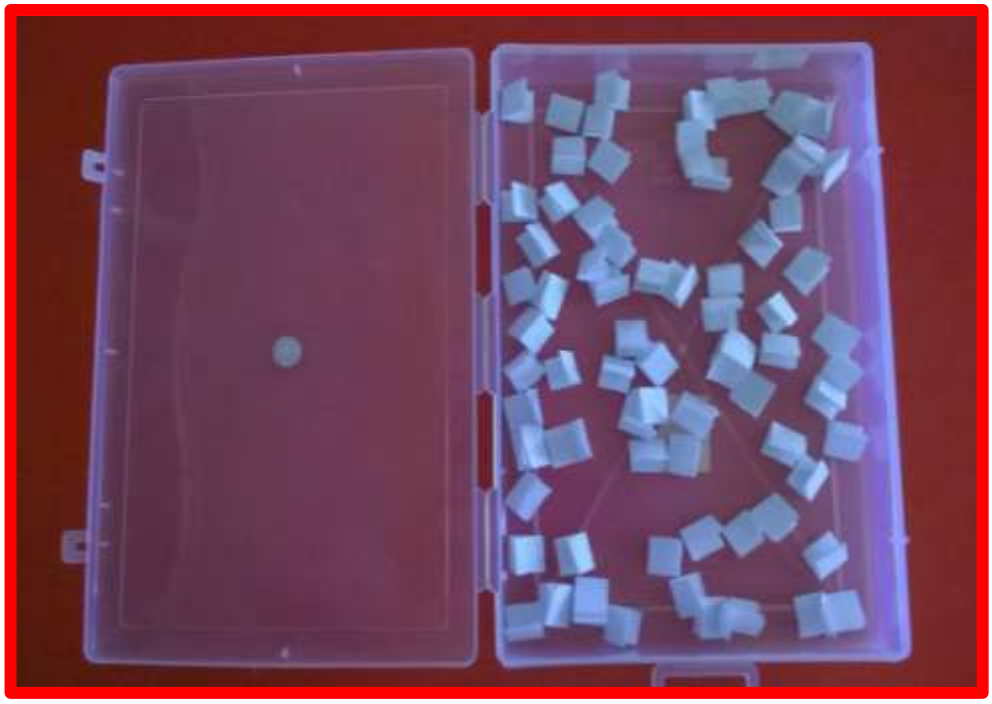


FIGURE 1: *Lottery method was used to narrow down to Thiagarajar college of Preceptors and Senthamil college, Madurai from a list of 70 different colleges in and around Madurai, which was obtained from the Government website.*

FIGURES 2 AND 3*: Pilot study was conducted at Thiagarajar college of Preceptors (left) and main study at Senthamil college, Madurai.(right)*

FIGURE 4: *Green tea leaves*

FIGURE 5: *2 grams of Green tea leaves measured using electronic weighing machine.*

FIGURE 6 AND 7*: Water heated in the heating mantle to which Green tea leaves were soaked for ten minutes. this mixture was filtered using filter paper.*

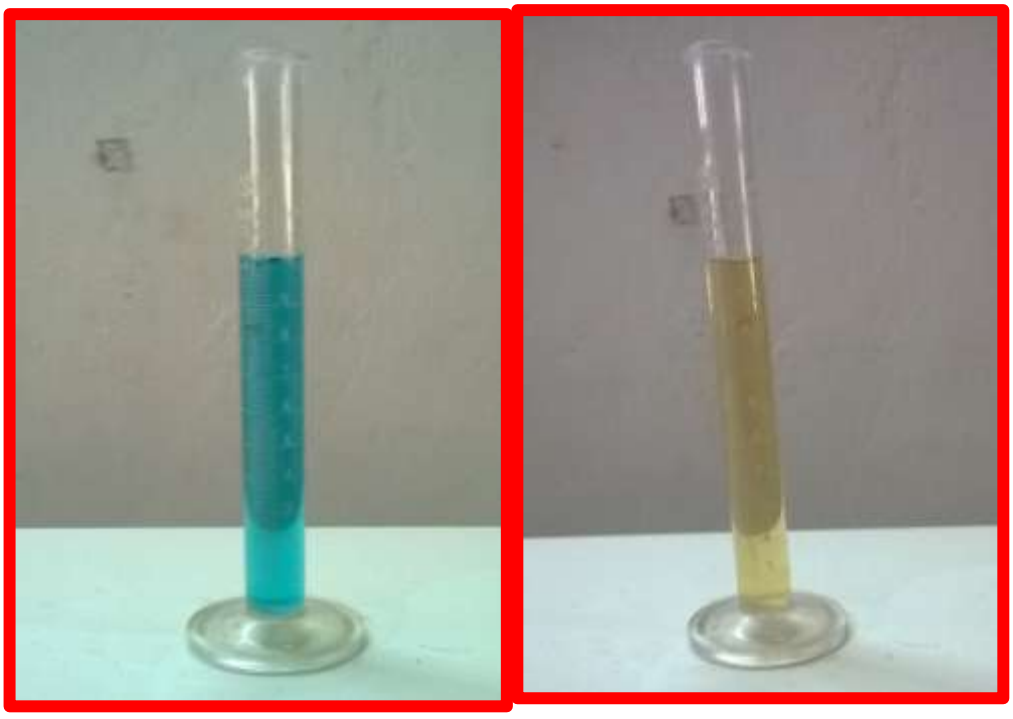


FIGURE 8 AND 9*: 10 ml of 0.2% Chlorhexidine and 2% Green tea extract was measured in measuring cylinder.*

FIGURE 10: *Blinding – letters with the codes of the intervention – in sealed envelopes*

FIGURE 11: *Opaque containers labelled and coded with respective interventions "X" (orange) and "Y" (yellow).*

FIGURE 12: *Randomization – box containing sealed envelopes with the codes "X" and "Y" for random allocation*

FIGURE 13: *Randomization – sealed envelopes opened by the principal investigator, randomly picked by the research participant, prior to allocation.*

FIGURE 14 AND 15*: Participants reading the patient information letter and signing the informed consent*

FIGURE 16*: Armamentarium for clinical examination*

FIGURE 17: *Clinical examination.*

FIGURE 18: *Identification cards with unique identification numbers, ready to be worn by the participants*

FIGURE 19: *Research participants seated in their respective seats prior to the start of the study.*

FIGURE 20: *(CLOCKWISE FROM LEFT) Materials used in the research – stopwatch, cold storage box, box with sealed envelopes, sucrose containers, intervention containers, paper towels, sterilized rubber bands, identification badges, table boards with identification codes, oral hygiene kits.*

FIGURE 21*: Cold storage box with saliva samples and ice packs*

FIGURE 22: *Sterilised rubber bands for chewing, to stimulate whole saliva packed in sterilised pouches*

FIGURE 23: *Sterile labelled containers for collection of stimulated saliva with stopwatch at the background*

***FIGURE 24**: Administration of the intervention*

***FIGURE 25**: Saliva collection by "spitting" method.*

FIGURE 26: *pH strips with holders - determination of salivary pH.*

FIGURE 27: *Determination of salivary pH. The changed colour of the pH strips were matched against the colour codes.*

FIGURE 28: *Culture media for Mutans Streptococci. M259-Mitis salivarius agar with 1% Potassium tellurite (Himedia, batch number: 228506) CMS208 bacitracin (Himedia, batch number: 249178).*

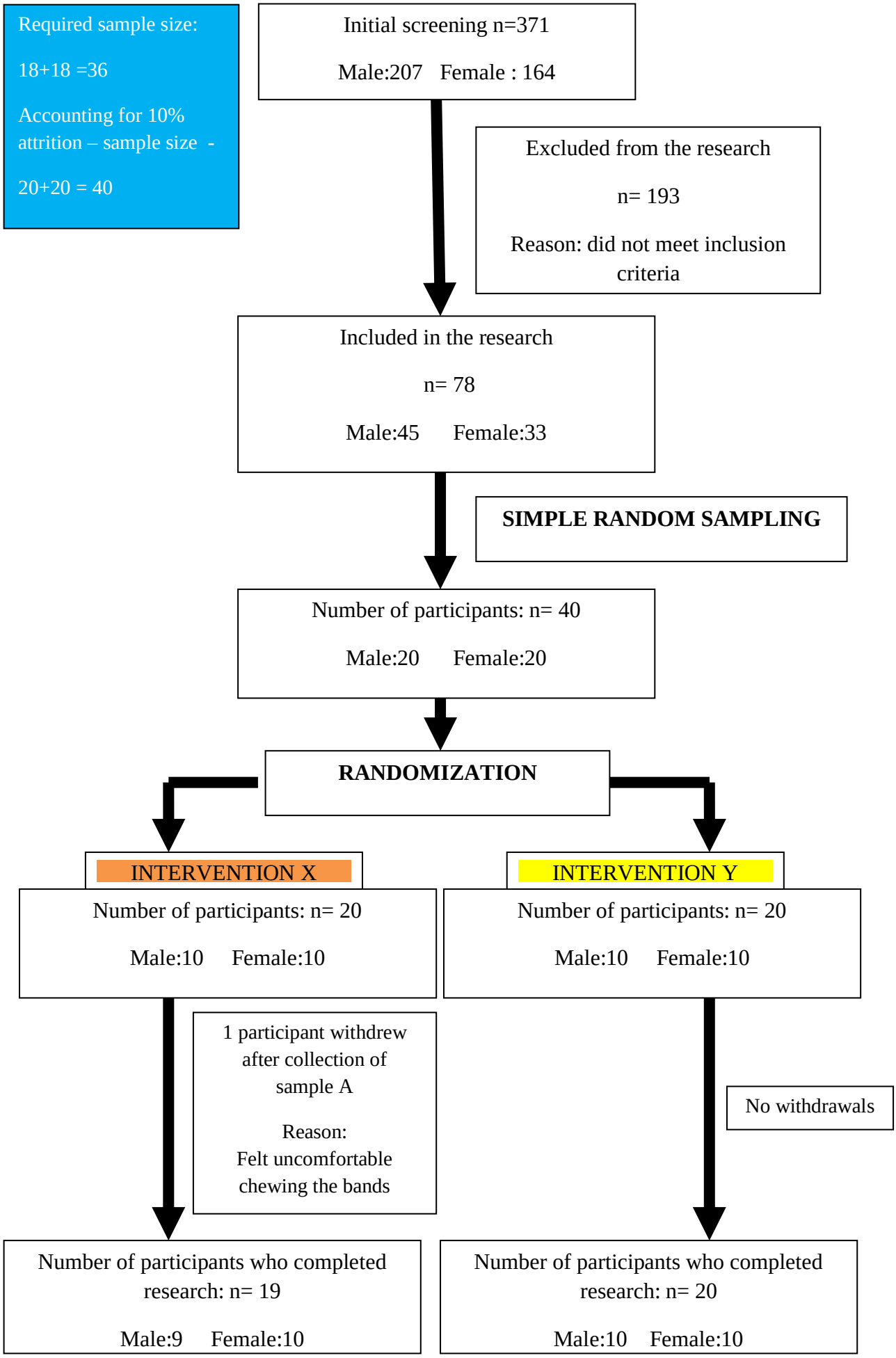
FIGURE 29: *Mutans Streptococci colonies cultured in the Mitis salivarius Bacitracin agar*

FIGURE 30: *Health education given to all the participants at the end of the research.*

FIGURE 31: *Oral hygiene kits were given to all participants*

Results

Flow of participants- CONSORT flowchart



RESULTS

The present research was a randomized, parallel arm, controlled trial designed to compare the effect of 2% Green tea extract with 0.2% Chlorhexidine mouthrinse on the Mutans Streptococci count in the saliva and salivary pH.

The main study was conducted among the college going students who were 17 – 25 years old attending Senthamil College, Madurai.

A total of 371 students in the college who were present on the day of the initial screening were clinically examined. This included 207 male patients and 164 female patients. Out of these students, 193 students did not fulfill the eligibility criteria were excluded at this stage. Only those who met the inclusion criteria (n=78) were recruited into the study at this stage. This included 45 male and 33 female students. “A master list” was prepared with the names of these 78 students and from this list 40 students were randomly picked on the day of research. This included 20 male and 20 female students. By stratified randomization students were allocated into the 0.2% Chlorhexidine group and 2% Green tea group. Ten male and ten female students in each group participated in the research. One male participant withdrew from the research during the course, after the collection of sample “A” as he was not comfortable chewing the bands to stimulate saliva. Therefore, 19 students (9 male and 10 female students) in 0.2% Chlorhexidine group and 20 students (10 male and 10 female students) in 2% Green tea group completed the research. Attrition was 2.5% in this present research. Sample size was already adjusted for a 10% attrition.

DEMOGRAPHIC CHARACTERISTICS

Age Distribution of the study participants in both the groups (TABLE 1)

A total of 40 participants were included in the study with respect to the sample size. Sociodemographic data revealed that the mean age of the study subjects was 19.7 years in 0.2% Chlorhexidine Group and 19.4 in 2% Green tea group with age ranging from 17-25 years. The 'p' value was not significant (p=0.5512).

Gender Distribution of the study participants in both the groups (GRAPH 1)

With respect to the gender of the participants, there was an equal distribution between the groups with n=10(50%) male subjects and n=10(50%) female subjects in each of the two groups. 'p' value was not significant (p=1.0)

Socio Economic Status of the study participants in both the groups (GRAPH 2)

The socioeconomic status of the study participants were calculated and were classified according to Kuppuswamy's socioeconomic status scale 2013.[27] Majority of the study subjects were in lower/upper lower class n=16 (80%) in 0.2% Chlorhexidine group and n=18(90%) in 2% Green tea group, followed by the middle/ lower middle class n=4 (20%) in 0.2% Chlorhexidine group and n=1 (5.0%) in 2% Green tea group and only one person n=1(5.0%) belonged to upper middle class in Green tea group. 'p' value was not significant (p=0.3307)

Decayed, Missing & Filled Teeth (DMFT) of the study participants in both the groups (TABLE 2)

DMFT scores were recorded using Henry.T.Klein, Carrole.E.Palmer And Knutson.J.W, 1938 Index. A majority of the study subjects had a DMFT score of 2, n=6 (30%) in both groups. n=5 (25%) participants and n=3 (15%) participants had DMFT more than 5 in 0.2% Chlorhexidine group and 2% Green tea group respectively. A mean DMFT score of 2.41 was observed among study subjects in 0.2% Chlorhexidine group and 1.96 in 2% Green tea group subjects. 'p' value was not significant (p=0.5688).

Mean Decayed, Missing & Filled Surfaces (DMFS) of the study participants in both the groups (TABLE 3)

DMFS scores were recorded using Henry.T.Klein, Carrole.E.Palmer And Knutson.J.W , 1938 Index. A mean DMFS score of 4.35 (S.D=3.84) was observed among study subjects in 0.2% Chlorhexidine group and 6.0 (S.D=4.22) in 2% Green tea group subjects. 'p' value was not significant (p=0.2037).

Mean Mutans Streptococcus Count (CFU/ml) in 0.2% CHLORHEXIDINE GROUP (TABLE 4 AND GRAPH 3)

In 0.2% Chlorhexidine group, The mean Mutans streptococci count (CFU/ml) of sample A was 92950 (SD=23716). There was a decrease in this mean CFU/ml in sample B after the 2 minute sucrose rinse 88850 (SD=25707). But 'p' value was not significant (p=0.6032). After one hour, post-rinsing with water, in sample C, there was a further decrease in the mean CFU/ml 87350(SD=28286). But 'p' value was not significant (0.8616). After the administration of the 0.2% Chlorhexidine there was no

growth of the microorganism in sample D. There was a statistical significance of the decrease in the mean CFU/ml between sample A and sample D (p=0.001), sample B and sample D (p=0.001), sample C and sample D (p=0.001).

Association between Mutans Streptococcus Count (CFU/ml) and Age / SES/ DMFT and DMFS scores in 0.2% CHLORHEXIDINE GROUP (TABLE 5)

In 0.2% Chlorhexidine group, When Age, Socioeconomic Status, DMFT and DMFS were correlated with the mean Mutans Streptococci count of each of the sample A,B,C and D to know the relationship between the variables, low positive correlation was observed between DMFT/DMFS and the CFU/ml which was suggestive of weak relationship between the variables. There was a low negative correlation between SES and CFU/ml suggestive of a weak relationship.

Mutans Streptococcus Count (CFU/ml) in 2% GREEN TEA GROUP (TABLE 6 AND GRAPH 4)

In 2% Green tea group, The mean Mutans streptococci count (CFU/ml) of sample A was 88200 (SD=21913). There was a slight increase in this mean CFU/ml in sample B after the 2 minute sucrose rinse 88250 (SD=21769). But 'p' value was not significant (p=0.9943). After one hour, post-rinsing with water, in sample C, there was a decrease in the mean CFU/ml 77000 (SD=27883) But 'p' value was not significant (p=0.1631). After the administration of the 2% Green tea the mean CFU/ml in sample D is 61150 (SD=31671). There was a statistical significance of the decrease between sample A and sample D (p=0.0033), sample B and sample D (p=0.0031). But the decrease in CFU/ml between sample C and sample D was not significant (p=0.1012).

Association between Mutans Streptococcus Count (CFU/ml) and Age / SES/ DMFT and DMFS in 2% GREEN TEA GROUP (TABLE 9)

In 2% Green tea group, When Age, SES, DMFT and DMFS were correlated with the mean Mutans Streptococci count of each of the sample A,B,C and D to know the relationship between the variables, mostly slight to low positive correlation was observed between DMFT/DMFS and the CFU/ml which was suggestive of weak relationship between the variables. There was a slight to low positive correlation between SES and CFU/ml suggestive of a weak or negligible relationship.

Mean Mutans Streptococcus Count (CFU/ml) in 0.2% CHLORHEXIDINE GROUP and 2% GREEN TEA GROUP (TABLE 7 AND GRAPH 7)

When comparing the Mutans Streptococcus Count (CFU/ml) in 0.2% Chlorhexidine group and 2% Green tea group, there was no significant difference in the values in sample A,B and C. Significant difference was observed between the Mutans Streptococcus Count (CFU/ml) in 0.2% Chlorhexidine group and 2% Green tea group in sample D alone (p=0.001).

Mean CFU differences between Sample B and Sample D in both groups (TABLE 8 AND GRAPH 9)

Significant difference was observed between the Mutans Streptococcus Count (CFU/ml) differences between B and D, in 0.2% Chlorhexidine group and 2% Green tea group (p= 0.001).

Mean pH of the samples in 0.2% CHLORHEXIDINE GROUP (TABLE 10 AND GRAPH 5)

In 0.2% Chlorhexidine group, the mean pH of sample A was 7.38(SD=0.39). There was a fall in this mean pH in sample B after the 2 minute sucrose rinse 6.58 (SD=1.62). The 'p' value was significant (0.039). After one hour, post-rinsing with water, in sample C, there was a rise in the mean pH 6.83(SD=1.66) But 'p' value was not significant (0.633). After the administration of the 0.2% Chlorhexidine group the mean pH in sample D is 6.95(SD=1.7). There is no statistical significance of the difference between sample A and sample D (p= 0.283), sample B and sample D (p= 0.48) and sample C and sample D (p= 0.816).

Mean pH of samples in 2% GREEN TEA GROUP. (TABLE 11 AND GRAPH 6)

In 2% Green tea group, The mean pH of sample A was 7.25(SD=0.44). There was a fall in this mean pH in sample B after the 2 minute sucrose rinse 6.95 (SD=0.43). The 'p' value was significant (0.036). After one hour, post-rinsing with water, in sample C, there was a rise in the mean pH 7.2(SD=0.3) The 'p' value was significant (0.038). After the administration of the 2% Green tea the mean pH in sample D was 7.25(SD=1.7). There was no statistical significant difference between sample A and sample D (p= 0.1) and sample C and sample D (p= 0.679). There was a statistical significant difference in the mean pH between sample B and sample D (p= 0.48).

Mean pH values in 0.2% CHLORHEXIDINE GROUP and 2% GREEN TEA GROUP (TABLE 12 AND GRAPH 8)

When comparing the salivary pH in 0.2% Chlorhexidine group and 2% Green tea group, there was no significant difference in the values in sample A,B,C and D.

Mean pH differences between Sample B and Sample D in both groups. (TABLE 13 AND GRAPH 10)

Significant difference was not observed between the pH value differences between samples B and D, in 0.2% Chlorhexidine group and 2% Green tea group ($p = 0.574$).

Table 1 : Age Distribution of the study participants in both the groups

Age Group	0.2% CHLORHEXIDINE GROUP		2% GREEN TEA GROUP	
	n	%	n	%
Up to 20 yrs	14	70.0	17	85.0
Above 20 yrs	6	30.0	3	15.0
Total	20	100.0	20	100.0
Mean	19.7 yrs		19.4 yrs	
SD	1.7 yrs		2.0 yrs	
'p'value*	0.5512** Not Significant			

***Chi square test **p>0.05**

Table 2 : Decayed, Missing & Filled Teeth (DMFT) of the study participants in both the groups

Decayed, Missing & Filled Teeth (DMFT)	0.2% CHLORHEXIDINE GROUP		2% GREEN TEA GROUP	
	n	%	n	%
1	5	25.0	1	5.0
2	6	30.0	6	30.0
3	-	-	2	10.0
4	4	20.0	5	25.0
5	-	-	3	15.0
Above 5	5	25.0	3	15.0
Total	20	100.0	20	100.0
Mean	3.4		3.8	
SD	2.41		1.96	
'p'value*	0.5688 Not Significant**			

***Chi square test **p > 0.05**

Table 3: Mean Decayed, Missing & Filled Surfaces (DMFS) of the study participants in both the groups

Group	Decayed, Missing & Filled Surfaces (DMFS)	
	Mean	S.D.
0.2% CHLORHEXIDINE GROUP	4.35	3.84
2% GREEN TEA GROUP	6.0	4.22
'p'value*	0.2037 Not significant**	

***Unpaired t test **p > 0.05**

Table 4: Mean Mutans Streptococcus Count (CFU/ml) in 0.2% CHLORHEXIDINE GROUP

Groups in 0.2% CHLORHEXIDINE GROUP	**Mutans Streptococcus Count (CFU/ml)**	
	Mean	**S.D.**
SAMPLE A (Baseline)	92950	23716
SAMPLE B (After Sucrose)	88850	25707
SAMPLE C (Baseline After 1 Hour)	87350	28286
SAMPLE D (After Intervention And Sucrose)	0	0
'p' value* between Groups		
A & B	0.6032 Not significant**	
A & C	0.5016 Not significant**	
A & D	**0.001 Significant*** **	
B & C	0.8616 Not significant**	
B & D	**0.001 Significant** ***	
C & D	**0.001 Significant*** **	

***Paired t test** ** **p > 0.05** ***p<0.05**

Table 5:Association between Mean Mutans Streptococcus Count (CFU/ml) and Age / SES/ DMFT and DMFS scores in 0.2% CHLORHEXIDINE GROUP

Variable	Correlation coefficient with Mutans Streptococcus Count in 0.2% CHLORHEXIDINE GROUP			
	Sample A	Sample B	Sample C	Sample D
Age	-0.014	0	0.066	-
SES	-0.384	-0.268	-0.403	-
DMFT	0.271	0.302	0.305	-
DMFS	0.215	0.272	0.264	-

Pearson's correlation coefficient test used.

Table 6: Mean Mutans Streptococcus Count (CFU/ml) in 2% GREEN TEA GROUP

Groups in 2% GREEN TEA GROUP	Mutans Streptococcus Count (CFU/ml)	
	Mean	**S.D.**
SAMPLE A (Baseline)	88200	21913
SAMPLE B (After Sucrose)	88250	21769
SAMPLE C (Baseline After 1 Hour)	77000	27883
SAMPLE D (After Intervention And Sucrose)	61150	31671
'p' value between Groups*		
A & B	0.9943 Not significant**	
A & C	0.166 Not significant**	
A & D	**0.0033 Significant*** **	
B & C	0.1631 Not significant**	
B & D	**0.0031 Significant*** **	
C & D	0.1012 Not significant**	

***Paired t test** ** p > 0.05** ***p<0.05**

Table 7: Mean Mutans Streptococcus Count (CFU/ml) in 0.2% CHLORHEXIDINE GROUP and 2% GREEN TEA GROUP

SAMPLES	Mutans Streptococcus Count (CFU/ml) in				'p'value*
	0.2% CHLORHEXIDINE GROUP		2% GREEN TEA GROUP		
	Mean	**S.D.**	**Mean**	**S.D.**	
Sample A	92950	23716	88200	21913	0.861 Not significant **
Sample B	88850	25707	88250	21769	0.846 Not significant**
Sample C	87350	28286	77000	27883	0.357 Not significant**
Sample D	0	-	61150	31671	**0.001 Significant*** **

***Unpaired t test** ** p > 0.05** ***p<0.05**

Table 8: Mean CFU differences between Sample B and Sample D in both groups

Variable	Mean CFU differences between Sample B and Sample D				'p'value*
	0.2% CHLORHEXIDINE GROUP		2% GREEN TEA GROUP		
	Mean	S.D.	Mean	S.D.	
Mutans Streptococci CFU/ml	88850	25707	27150	34586	0.001 Significant***

*Unpaired t test ** p > 0.05 ***p<0.05

Table 9:Association between Mutans Streptococcus Count CFU/ml and Age / SES/ DMFT and DMFS in 2% GREEN TEA GROUP

Variable	Correlation coefficient with Mean Mutans Streptococcus Count in 2% GREEN TEA GROUP			
	Sample A	Sample B	Sample C	Sample D
Age	-0.039	0.127	0.121	0.128
SES	0.157	0.151	0.241	0.396
DMFT	-0.118	0.133	0.272	0.365
DMFS	0	0.195	0.237	0.199

Pearson's correlation coefficient test used.

Table 10: Mean pH of the samples in 0.2% CHLORHEXIDINE GROUP

Groups in 0.2% CHLORHEXIDINE GROUP	pH	
	Mean	**S.D.**
SAMPLE A (Baseline)	7.38	0.39
SAMPLE B (After Sucrose)	6.58	1.62
SAMPLE C (Baseline After 1 Hour)	6.83	1.66
SAMPLE D (After Intervention And Sucrose)	6.95	1.7
'p' value between Groups*		
A & B	**0.039 Significant*** **	
A & C	0.159 Not significant**	
A & D	0.283 Not Significant**	
B & C	0.633 Not significant**	
B & D	0.48 Not Significant **	
C & D	0.816 Not Significant**	

***Paired t test** ** p > 0.05** ***p<0.05**

Table 11: Mean pH of samples in 2% GREEN TEA GROUP.

Groups in 2% GREEN TEA GROUP	pH	
	Mean	**S.D.**
SAMPLE A (Baseline)	7.25	0.44
SAMPLE B (After Sucrose)	6.95	0.43
SAMPLE C (Baseline After 1 Hour)	7.2	0.3
SAMPLE D (After Intervention And Sucrose)	7.25	0.44
'p' value between Groups*		
A & B	**0.036 Significant*** **	
A & C	0.679 Not significant**	
A & D	1.0 Not Significant**	
B & C	**0.038 Significant*** **	
B & D	**0.036 Significant*** **	
C & D	0.679 Not significant**	

***Paired t test** ** p > 0.05** ***p<0.05**

Table 12: Mean pH values in 0.2% CHLORHEXIDINE GROUP and 2% GREEN TEA GROUP

SAMPLES	pH values in				'p'value*
	0.2% CHLORHEXIDINE GROUP		2% GREEN TEA GROUP		
	Mean	S.D.	Mean	S.D.	
Sample A	7.38	0.39	7.25	0.44	0.352 Not significant**
Sample B	6.58	1.62	6.95	0.43	0.324 Not significant**
Sample C	6.83	1.66	7.2	0.3	0.328 Not significant**
Sample D	6.95	1.7	7.25	0.44	0.45 Not significant**

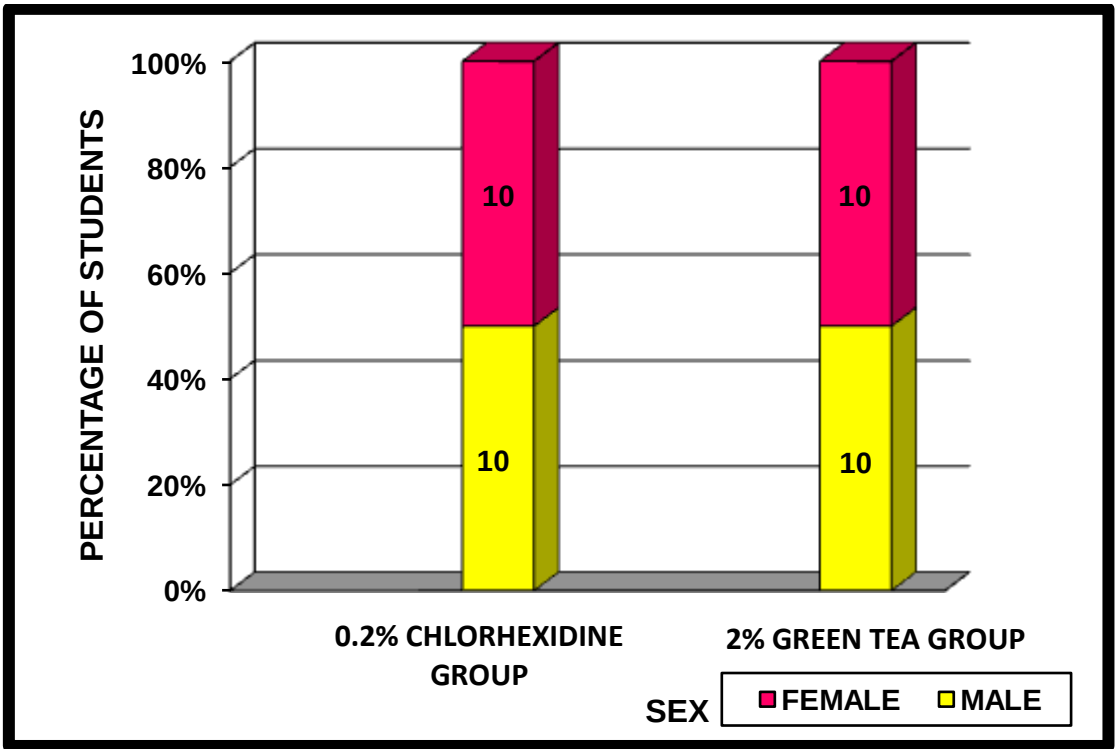
***Unpaired t test** **** p > 0.05** *****p<0.05**

Table 13: Mean pH differences between Sample B and Sample D in both groups.

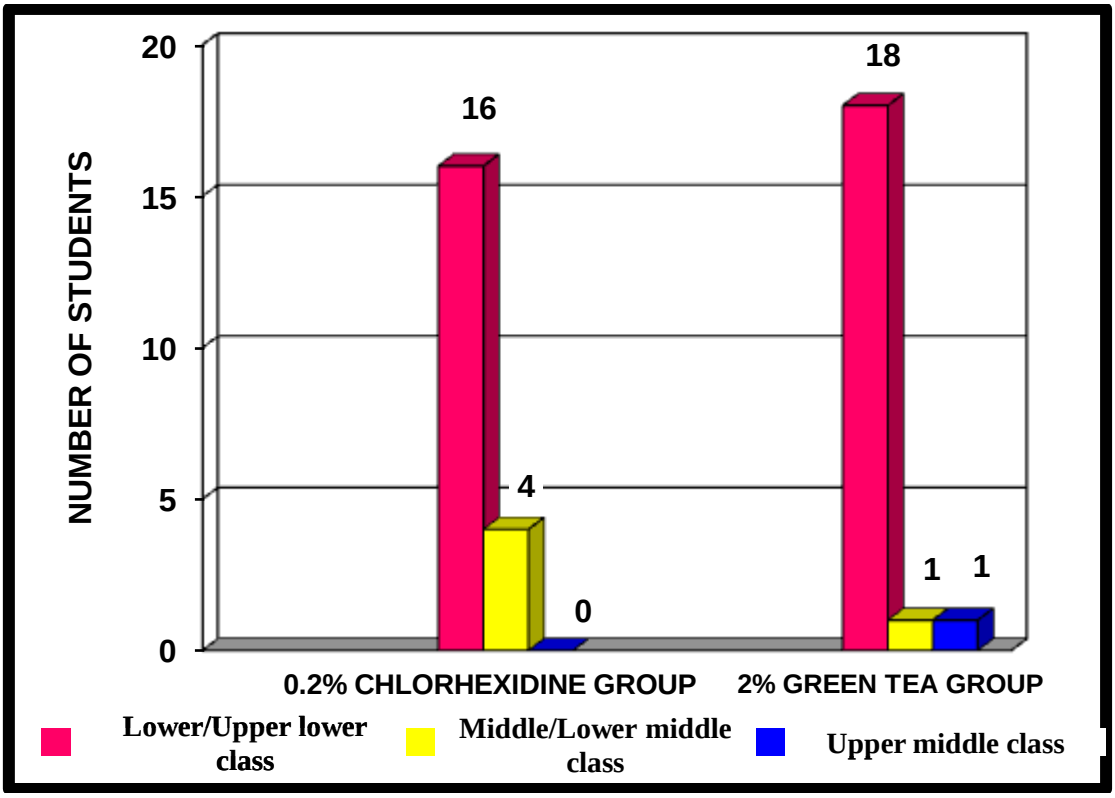
Variable	Mean pH differences between Sample B and Sample D				'p'value*
	0.2% CHLORHEXIDINE GROUP		2% GREEN TEA GROUP		
	Mean	S.D.	Mean	S.D.	
pH	0.375	0.395	0.3	0.44	0.574 Not significant**

***Unpaired t test** **** p > 0.05** *****p<0.05**

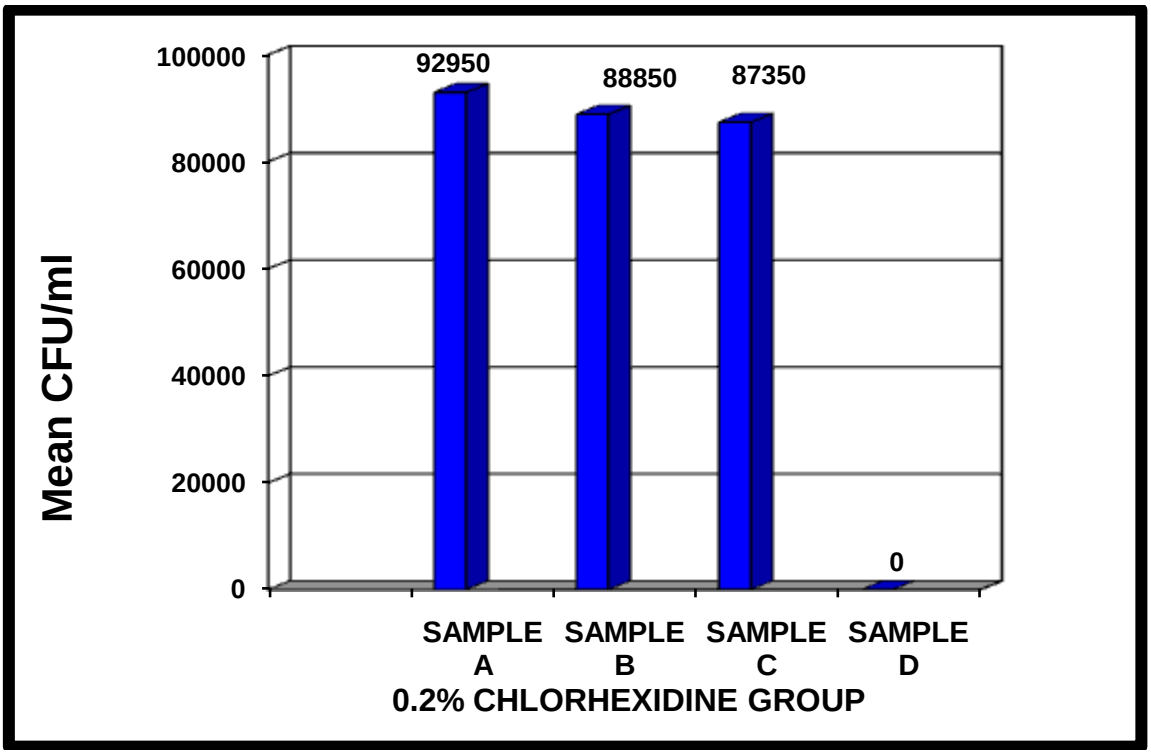
GRAPH 1 : Gender Distribution of the study participants in both the groups



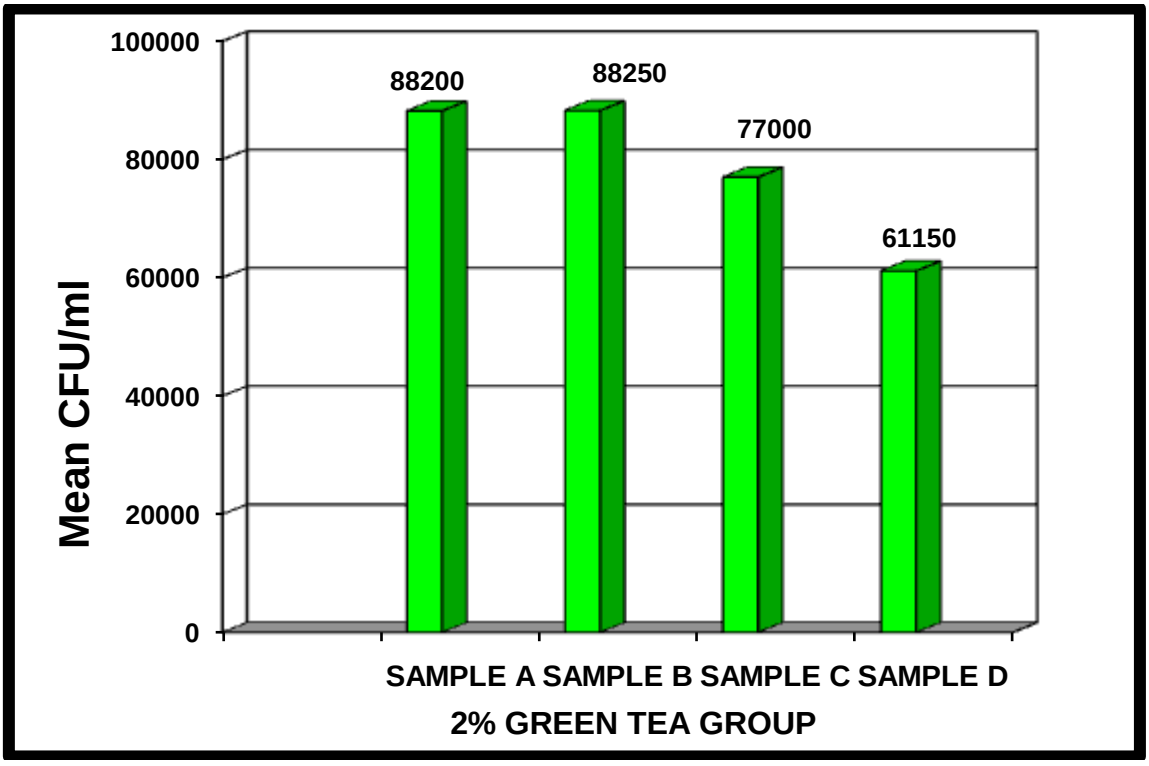
GRAPH 2 : Socio Economic Status of the study participants in both the groups



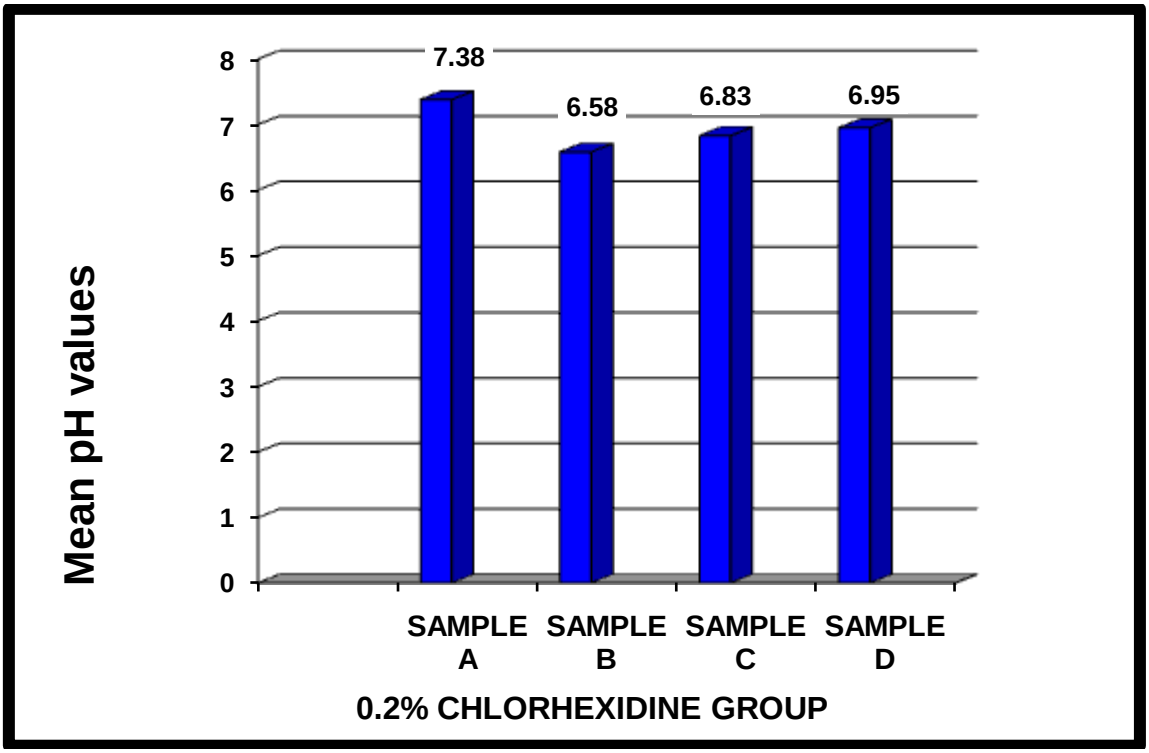
GRAPH 3: Mean Mutans Streptococcus Count (CFU/ml) in 0.2% CHLORHEXIDINE GROUP



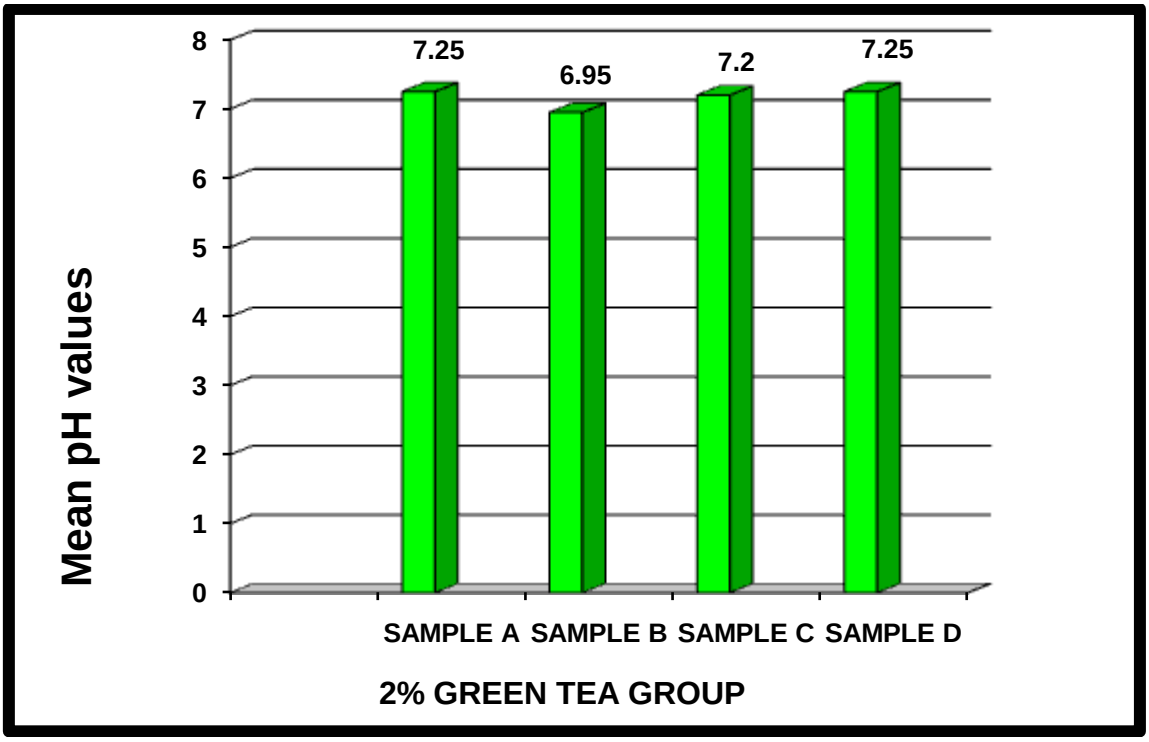
GRAPH 4: Mean Mutans Streptococcus Count (CFU/ml) in 2% GREEN TEA GROUP



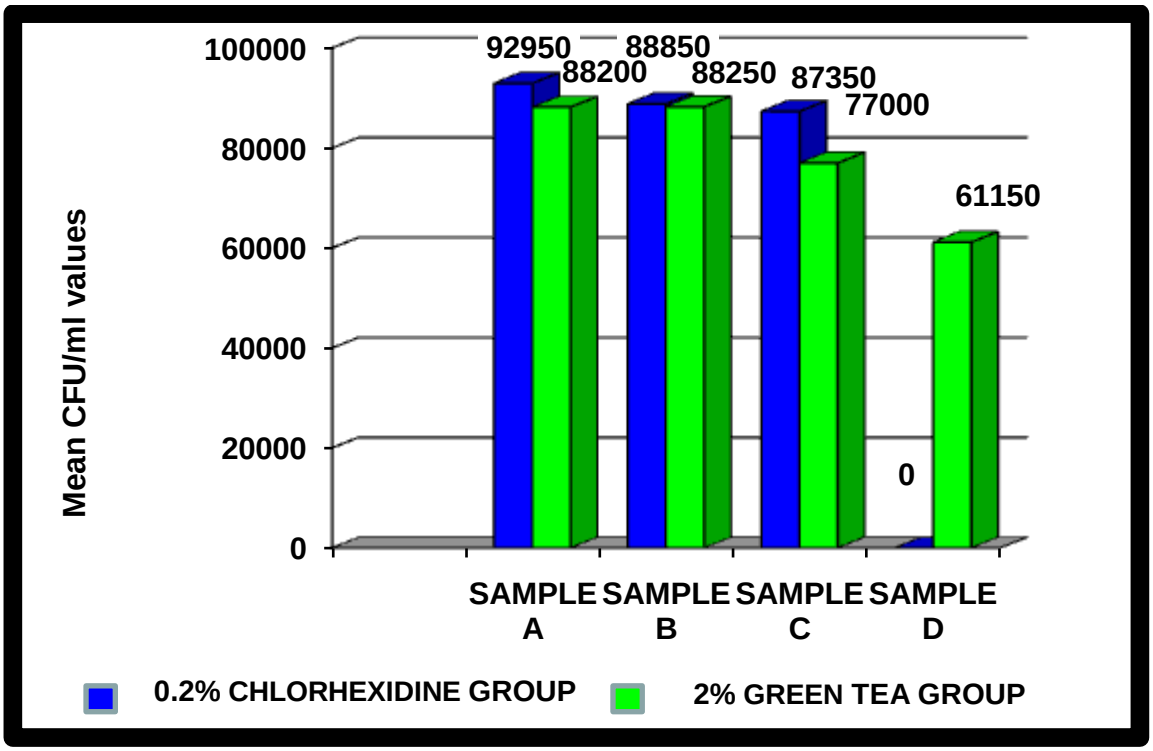
GRAPH 5: Mean pH values of the samples in 0.2% CHLORHEXIDINE GROUP



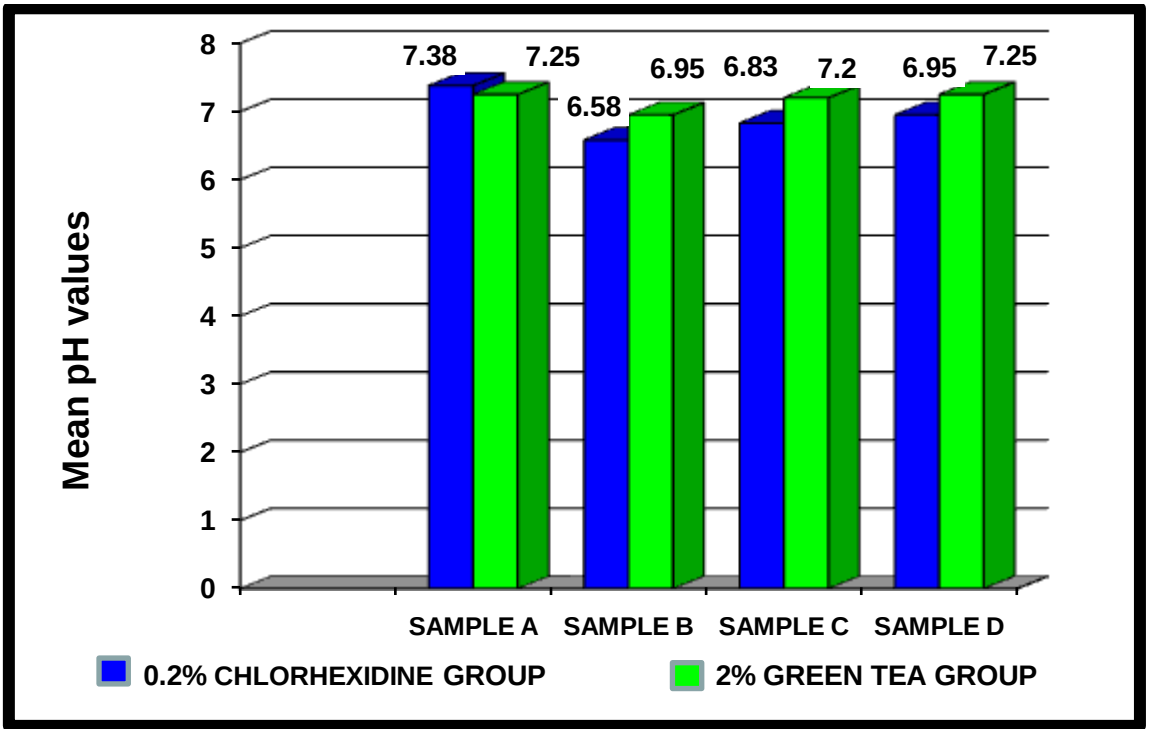
GRAPH 6: Mean pH values of the samples in 2% GREEN TEA GROUP



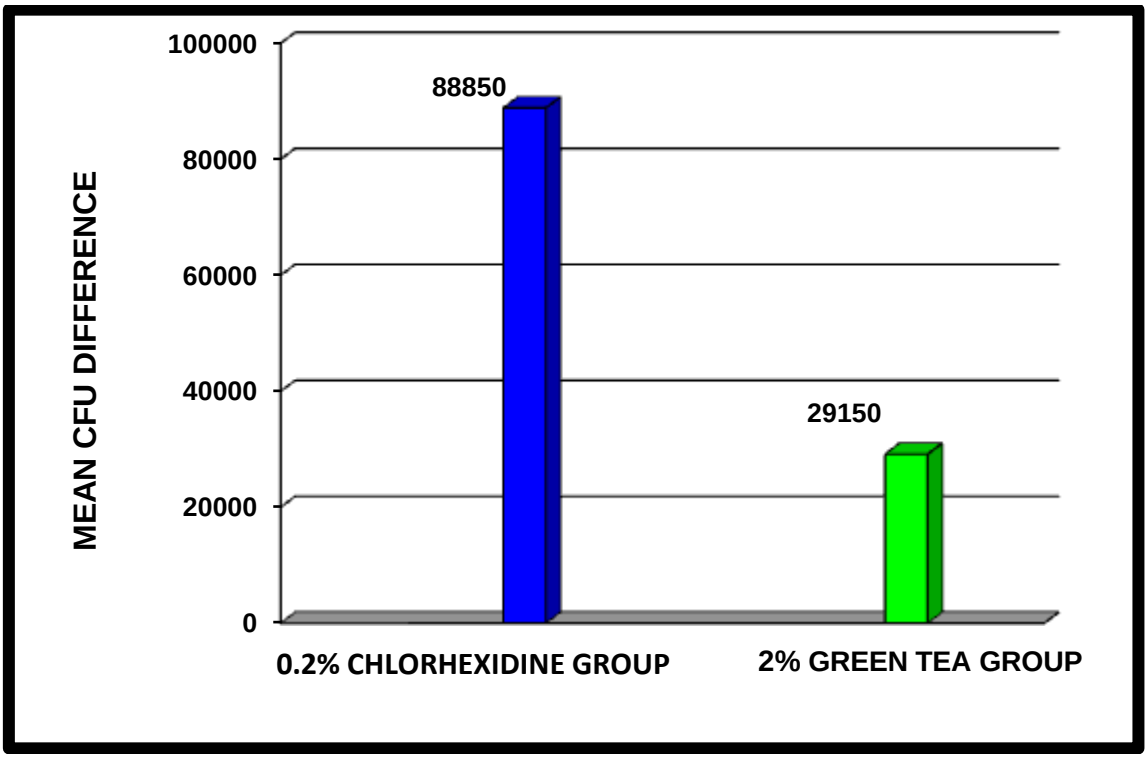
GRAPH 7: Mean Mutans Streptococcus Count (CFU/ml) in 0.2% CHLORHEXIDINE GROUP and 2% GREEN TEA GROUP



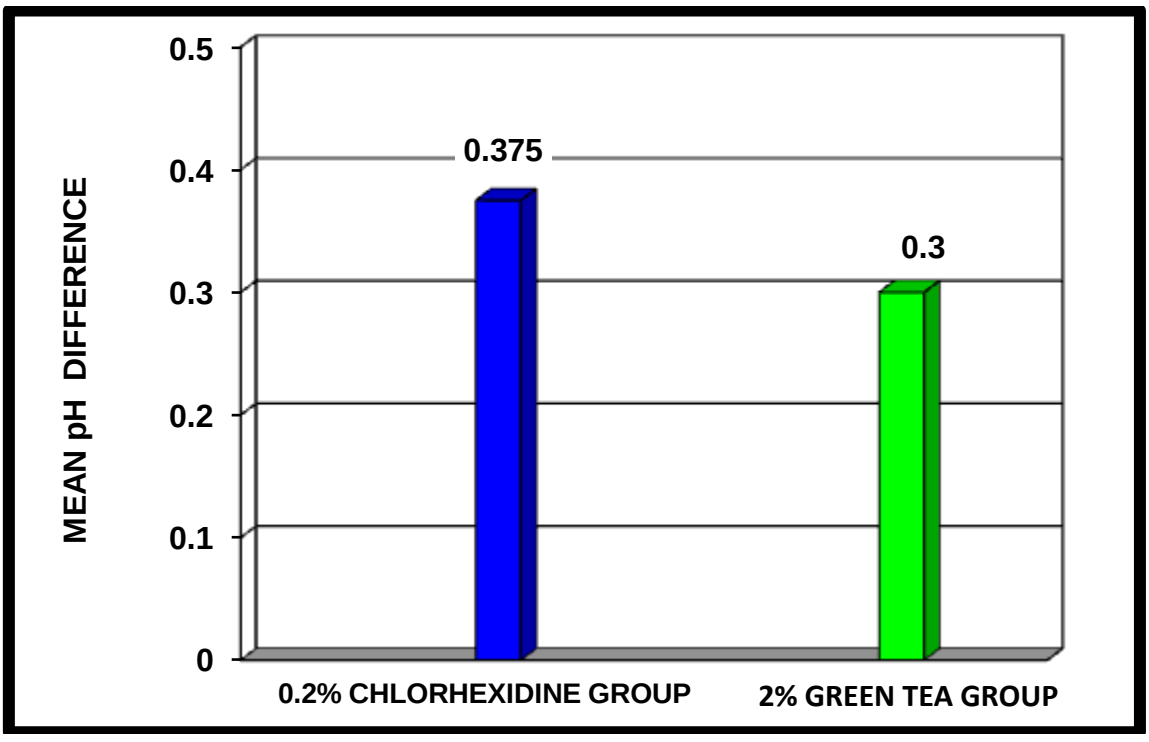
GRAPH 8:Mean pH values of the samples in 0.2% CHLORHEXIDINE GROUP and 2% GREEN TEA GROUP



GRAPH 9: Mean CFU differences between Sample B and Sample D in both groups



GRAPH 10: Mean pH differences between Sample B and Sample D in both groups.



Discussion

DISCUSSION

Dental caries is claimed to be an ubiquitous and almost universal bacterial infection that has been afflicting mankind since the days of civilization. It is also hailed as an expensive disease, owing to the cost in terms of money spent for treatment as well as the human suffering it wroughts. Ever since Keyes in 1969, stated that the caries inducing factors are the teeth, the oral microflora and the dietary substrates, it deemed fit to call dental caries as a "multifactorial disease".[31] Thus began the battle against caries, with the identification of the predisposing factors that caused this disease. Approaches that combated each of these factors, like increasing the resistance of the host, eliminating the cariogenic micro-organisms and modifying the diet were adopted.[8]

The pathogenesis of caries involves the synthesis of sticky glucans by the oral microbes, adherence of bacteria to the hard tissues and formation of plaque, and the acid production at this tooth-plaque interface. The last two steps however require a substrate like sucrose – "the arch-criminal of dental caries."[32] Theoretically speaking, inhibition of each of these steps leads to the prevention of dental caries. Mechanical plaque control is said to be the best approach to prevent biofilm formation and chemotherapeutic agents can be used as an effective adjunct for this purpose.[21]

Although Miller's chemicoparasitic theory incriminated dental plaque to be a causative factor of dental caries in the early 20th century, researchers had already started a laborious search for the specific elusive cause of dental caries. Researches pointed out to Mutans Streptococci in the oral cavity as the primary causative micro-organism of dental caries. Since then, various antimicrobial agents have been tried

and tested against these micro-organisms. Some of them have entered the markets while others remain at the research level.

Chlorhexidine is considered as the "gold standard" antimicrobial agent as it exhibits bacteriostatic properties at low concentrations and bactericidal properties at higher concentrations. It is used as a positive control in several studies that explore newer anticariogenic agents. However longterm use of Chlorhexidine is not advocated due to its side-effects like taste alterations, dental staining and mucosal erosions.[20]

Awareness about the side effects of chemical plaque control agents have brought to light several herbal products that have antibacterial properties. Some of them include, Guava (Psidium guajava), Garlic (Allium sativum), Neem (Azadirachta indica), Lime (Citrus aurantifolia), Pomegranate (Punica granatum), Green tea (Camellia sinensis), Tulsi (Ocimum sanctum), Cranberry (Vaccinium macrocarpon) etc.[33]

Of late, numerous medical research are done on green tea for its medical benefits. Green tea is attributed with beneficial properties like anti-tumorogenic property, antioxidant property, hypolipidemic property, antimutagenic and anti carcinogenic property, antifungal property, antibacterial effects and neuroprotective properties which have been substantiated by lab and animal studies. Japanese folklore expound that consumption of green tea makes the mouth clean and makes it less vulnerable to tooth decay.[34] Tea is the second most popular drink next to water. Tea leaves from the plant Camellia sinensis are of 3 types depending on the type of processing. The active ingredient in green tea are the polyphenols namely, catechin, epicatechin, gallocatechin (GC), epicatechin gallate, epigallocatechin (EGC) and epigallocatechin gallate (EGCg).[10]

Available literature shows that there are few studies comparing the efficacy of Chlorhexidine with Green tea as an antimicrobial agent.[10]

The present research is a randomized, parallel arm, controlled trial designed to compare the effect of 2% Green tea extract with 0.2% Chlorhexidine mouthrinse on the Mutans Streptococci count in the saliva and salivary pH.

The present study closely but not entirely follows the methodology of a previous study done by Awadalla et al, in which the antibacterial efficacy of 2% Green tea is assessed.[9] In contrast, The present study compares the antibacterial efficacy of 2% Green tea with that of 0.2% Chlorhexidine. Baseline collection of salivary samples is an additional component to the methodology. Therefore accurate comparisons are not possible. An attempt has been made to make valid comparisons of selected results wherever possible.

REASON FOR CHOOSING MUTANS STREPTOCOCCI COUNT IN SALIVA AS THE PRIMARY OUTCOME

Mutans Streptococci are the surrogate marker for dental caries as they are implicated to be the initiators of the disease and their quantity in the saliva is directly related to the number of surfaces colonized by them.[29] Any intervention that can hamper their growth and survival will negatively impact the initiation and progress of caries. Mutans Streptococci also play an important role in caries prediction. Individuals who are heavily colonized by these microbes are classified to be at high risk for caries.[30] They can adhere to the oral hard tissues by producing sticky dextrans by glucosyl transferase activity on sucrose.

REASON FOR CHOOSING SALIVARY pH AS THE PRIMARY OUTCOME:

The pH in the oral cavity is an indirect measure of the number of acid producing bacteria – Mutans Streptococci in the oral cavity, or a measure of their metabolism that converts sugars into the acids.[1] The virulence factor of Mutans Streptococci include, tooth surface adhesion, acid production and acid tolerance. These bacteria rapidly metabolize sugars into lactic acid compared to other oral microbes. They also have the ability to thrive and continue their metabolism in this acidic environment. It is also important to note the time required to reach the critical pH (5.0-5.5) and the duration for which the pH remains close to it before it raises to the normal range to assess the efficacy of the caries preventive agents.

REASON FOR CHOOSING STIMULATED WHOLE SALIVA:

The participant chews the rubber band to transfer the bacteria from the tooth surfaces into the saliva. Whole saliva is a representative sample of not just the secretions from the major and minor glands but also microbes and their products, gingival exudates, epithelial cells and food debris. Whole saliva is said to be of clinical relevance for caries susceptibility and carious activity. With respect to stimulation, there is a huge increase in terms of salivary output, the consistency and the concentration of many of its ingredient such as proteins, immunoglobulins etc.[29]

DMFT/DMFS AND MUTANS STREPTOCOCCI COUNTS

In the present study no significant strong correlation was found between DMFT/DMFS scores and Mutans Streptococci counts. This is in accordance with Walter.J.Loesche, who has documented similar unimpressive correlations between these two morbidity indices and the bacterial levels. However, this might be attributed

to the insensitivity of the indices in classifying the caries active individuals and caries inactive individuals.[1]

SALIVARY PARAMETERS

MUTANS STREPTOCOCCUS COUNT (CFU/ml) AND SALIVARY pH IN 0.2% CHLORHEXIDINE GROUP

In this research, while comparing the baseline mean Mutans streptococci salivary counts (samples A, B, C) with that of the post-intervention rinse sample (Sample D) showed a significant reduction in the Mutans Streptococci colonies ($p<0.003$) (Table 4 and Graph 3). Thus it shows that 0.2% Chlorhexidine is potent antimicrobial agent against this group of bacteria. Also, Chlorhexidine showed the capability of maintaining the salivary pH near neutrality (6.95) even after a 10% sucrose challenge was administered. Though, the comparison between the baseline pH values (sample A, B, C) and post-intervention sample (sample D) showed an increase (Table 10 and Graph 5), this was not statistically significant.

This result is in accordance with previous studies that have demonstrated the antibacterial efficacy of Chlorhexidine.[3,15,17-20,35-37] These studies have either used 0.2% or 0.12% Chlorhexidine in their studies. In a cross over trial conducted in India by Neturi et al, 0.12% Chlorhexidine caused significant reductions in Mutans Streptococci.[17] Similarly, Faria et al in their study observed that 0.12% chlorhexidine reduced the Mutans Streptococci colonies significantly.[35] Fajriani et al in their in vivo research also showed that 0.2% Chlorhexidine significantly reduced the Mutans Streptococci colonies.[19]

The mechanism of action of Chlorhexidine in reducing the Mutans Streptococci count is by altering the bacterial cell membrane by attaching to the phospholipids which causes leakage of the intracellular potassium. The cytoplasm of the cell gets

precipitated. Substantivity or the oral retentiveness of Chlorhexidine is the another unique property that renders a longer lasting of this antibacterial effect upto several hours.[25,26] The duration of how much longer Chlorhexidine exerts this effect also depends on the extent to which the antimicrobial agent is held in the retentive areas in the oral cavity. However this study collected post-intervention samples at 30 minutes only.

However while comparing all the various forms of Chlorhexidine, varnishes seem to render the most persistent reduction of Mutans Streptococci, followed by gels and mouthrinses.[36]

Chlorhexidine also inhibits acid production by the oral micro-organism by negating the activity of phosphoenol pyruvate-phototransferase sugar transport system and this also is reported to be an important mode of exerting an caries-inhibitory effect.[26]

MUTANS STREPTOCOCCUS COUNT CFU AND SALIVARY pH IN 2% GREEN TEA GROUP

The present study investigated the effect of 2% Green tea extract as mouthrinse for 5 minutes on the Mutans Streptococci count (CFU/ml) in the saliva and the salivary pH after a 10% sucrose challenge which was rinsed for 2 minutes. The post-intervention sample (sample D) was taken after 7 minutes of this sucrose challenge. The results showed a decrease in the Mutans Streptococci count after the intervention and this was statistically significant. (p=0.003) (Table 6 and Graph 4). While comparing the salivary pH levels, it was shown that Green tea helped the pH to remain near neutrality (7.25) and this was statistically significant (p=0.036) (Table 11 and Graph 6) The decrease in the Mutans Streptococci count after the administration of Green tea is in accordance with previous studies.[6-11,14-15,18-19,35,37] However these studies have used various concentrations of Green tea

like 0.2%, 2.5%, 5%, 25% and 50-400 mg/ml of Green tea extract. Some studies have isolated the bioactive catechins and used them at varying concentrations like 10-100 micrograms/ml of isolated and purified polyphenols, 60mg/ml of Green tea polyphenols, and 2mg/ml of ECGg. But all these studies have unanimously shown that Green tea considerably reduces Mutans Streptococci counts both in vitro and in vivo. The in vitro study by Sakanaka et al showed that a five minute exposure to the polyphenols significantly reduced Streptococcus mutans count.[10] Similar results were obtained by Awadalla et al in the in vivo research which showed that rinsing with 2% Green tea significantly reduces Mutans Streptococci ($p<0.001$).[9] Otake et al in an in vivo research showed that the polyphenolic compounds were highly capable of reducing the Streptococcus mutans count.[8]

The inhibition of the fall in the salivary pH after the intervention is clearly demonstrated and is statistically significant. This is in accordance with previous study by Awadalla et al in which salivary and plaque pH were evaluated at 3, 7, 11, 20 and 30 minutes after 2% Green tea mouthrinse and 10% sucrose challenge. The study showed a decrease in plaque and salivary acidity.[9] This further reinstates the anticariogenic property of Green tea in maintaining the pH near neutrality. Hamilton Miller proposed that oral rinsing with sufficient quantity of Green tea extracts can inhibit acid production by bacteria which further does not act as a conducive environment for the survival of aciduric bacteria, thereby reducing the count.[34] Hirasawa et al in an *in vivo* research showed that a 2% Green tea rinse for 5 minutes was capable of maintaining the plaque pH within normal range at 3, 7, and 11 minutes post-rinsing, significantly.[11]

The active ingredients were revealed to be polyphenols like catechin, Epicatechin, Gallocatechin (GC), Epicatechin Gallate, Epigallocatechin (EGC)

and Epigallocatechin Gallate (EGCg). The MIC of the components GC, EGC, EGCg, is 250 micrograms/ml, 250 or 500 micrograms/ml, 500 - 1000 micrograms/ml respectively. The minimum time required for the bactericidal action of EGCg was thirty minutes, when the CFU/ml of Streptococcus mutans bacteria was reduced to one tenth of the initial value. In eight hours, the CFU/ml was reduced to almost a negligible value. Previous studies recommend that drinking one cup of Green tea a day, which is 100 ml, contains more polyphenols can definitely aid in the reduction of the incidence of Dental caries.[10]

The composition of Green tea is complex. It contains polyphenols, alkaloids, amino acids, carbohydrates, proteins, pigments, minerals and trace elements. Polyphenols are those bioactive substances that have been found to exert the anticariogenic effect of tea. The caries inhibitory effects of tea are :

1. **The direct bactericidal effect**: The polyphenols catechin, epicatechin, gallocatechin (GC), epicatechin gallate, epigallocatechin (EGC) and epigallocatechin gallate (EGCg) are present in Green tea. Although little is known about the exact mode of action, it is believd that polyphenols disrupt bacterial cell membranes.[34]
2. **Prevent bacterial adherence to teeth:** the polyphenols act by inhibiting the glucosyl transferase activity that converts sucrose to glucans. This sticky glucan helps in the adherence of bacteria to the tooth as well as the other micro-organism in the plaque.
3. **Reduce Biofilm formation:** by interfering with the bacterial adherence as explained above.
4. **Inhibition of salivary and bacterial amylases**: It help in reduced metabolism of starch in the oral cavity which explains the acid inhibition property.[34]

5. **Affinity for proteins:** The mechanism of action also proposed for antibacterial activity is also the competitive binding of the tannins to proline rich proteins in the saliva and the bacterial cell surfaces, thereby making them unavailable for bacterial adhesion.[8] The binding also causes distortion of tertiary structure of the bacteria and subsequently the loss of function.
6. **Fluoride in Green tea**: The fluoride content is also believed to exert a considerable cariostatic action. by inhibiting the demineralization of dentin in the caries process.[16] But the level of fluoride in tea was found to be too low (0.38 ppm to 0.70 ppm) and this fact suggests that it may not be the only caries inhibitory component.[15]

COMPARING THE EFFICACY OF 0.2% CHLORHEXIDINE AND 2% GREEN TEA

Significant difference was observed between the Mutans Streptococcus Count (CFU/ml) differences between B and D, in 0.2% Chlorhexidine group and 2% Green tea group (p= 0.001). The reduction in Mutans Streptococci count was considerable in the Green tea group and was statistically significant when compared to 0.2% Chlorhexidine, which was much superior in its efficacy.

This is in accordance with the previous literature that suggest that Chlorhexidine is superior to Green tea in the reduction of Mutans Streptococci.[17,18,20,35]

However, certain studies show that Green tea exhibits antibacterial efficacy comparable with Chlorhexidine.[19,37] Fajrani et al in their research found no significant difference in the effect of 0.2% Chlorhexidine and 2.5% green tea on the Mutans Streptococci count.[2] This study was conducted among 6-12 year old children in Indonesia. However in an *in vitro* study by Subramaniam et al in India, 50% aqueous extracts of Green tea showed superior reduction of Mutans Streptococci count

compared to 0.2% Chlorhexidine which was statistically significant.[15] The differences might be due to many reasons, one of it being the geographical location where the Green tea was grown. The catechin content in the green tea is bound to vary depending upon the soil, rainfall, climate, and the type of processing.[17] Also the difference in the concentration of the Green tea extract and the method of preparation, the differences in the study population that might contribute differences in the external modifying factors of dental caries like Dietary factors, Socioeconomic status and behavior, and Internal modifying factors of dental caries like saliva, general health and variations in tooth size, morphology, composition can contribute to the variability in results.[29] Therefore results may not be strictly compared. Few studies report the beneficial effects of Green tea on Periodontal health.[38-40] Few studies report side effects of Green tea too.[41-42]

Thus it shows that 2% Green tea was considerably efficacious in reducing the Mutans Streptococci count in comparison with the gold standard 0.2% Chlorhexidine, It proves to be a promising anticariogenic mouthrinse with its acid inhibition property in this research by maintaining the salivary pH near neutrality significantly.

LIMITATIONS OF THE STUDY

The age group for the present study is narrow and the study results can only be extrapolated to this particular age group and subjects who attend educational institutions in Madurai and therefore the generalizability is limited. This research included only one post-rinse salivary samples collected after 30 minutes of the intervention. Therefore long term or substantivity of the Green tea mouthrinse could not be compared with that of Chlorhexidine. Further long-term studies are recommended.

Summary and Conclusion

SUMMARY AND CONCLUSION

The present research is a randomized, parallel arm, controlled trial designed to compare the effect of 2% Green tea extract with 0.2% Chlorhexidine mouthrinse on the Mutans Streptococci count in the saliva and salivary pH. The results could be summarised as:

1. There was a statistically significant reduction in the Mutans Streptococci colonies in the saliva after 0.2% Chlorhexidine mouthrinse.
2. There was a statistically significant reduction in the Mutans Streptococci colonies in the saliva after 2% Green tea extract mouthrinse.
3. Significant difference was observed between the Mutans Streptococcus Count (CFU/ml) between the 0.2% Chlorhexidine group and 2% Green tea group.
4. There is a rise in the salivary pH towards neutrality after rinsing with the 0.2% Chlorhexidine mouthrinse but this is not statistically significant.
5. The inhibition of the fall in the salivary pH after 2% Green tea extract mouthrinse is clearly demonstrated and is statistically significant.

Thus we can conclude that,

2% Green tea was considerably efficacious in reducing the Mutans Streptococci count significantly, although not superior to the gold standard 0.2% Chlorhexidine. It proves to be a promising anticariogenic mouthrinse with its acid inhibition property in this research by maintaining the salivary pH near neutrality significantly, even after a sucrose challenge and can be used as a cost effective and safe caries inhibitory agent.

Recommendations

RECOMMENDATIONS

This research aspired to explore the efficacy of Green tea as a potential antibacterial mouthrinse and it was concluded that 2% Green tea was considerably efficacious in reducing the Mutans Streptococci count in comparison with the gold standard 0.2% Chlorhexidine. It proves to be a promising anticariogenic mouthrinse with its acid inhibition property.

However further research might aid in strengthening this evidence established in the study.

- Since the exact caries inhibitory mechanism of green tea is still not understood, future research to unveil the precise mode of action is advocated.
- Previous researches have pointed out the beneficial effects of Green tea on the periodontal health too. Therefore, possible application of this research to incorporate green tea in oral hygiene aids like toothpastes, chewing gums, mouthwashes can be explored.
- Very few trials have been conducted on Green tea. Though it comes with the aura of "being natural", there are studies reporting its side effects. Therefore longterm trials are recommended to establish its safety in human beings.
- Research to investigate the feasibility of incorporating of Green tea polyphenols in dental materials can be undertaken to best exploit its acid inhibition property.
- Studies using Economic analysis to weigh the cost-effectiveness of Green tea should also be done, to explore it as a public health intervention especially in a developing country like India.

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Annexures

LIST OF ANNEXURES

S.NO	ANNEXURES
1.	Ethical approval by Institutional Review Board
2.	Patient information letter
3.	Informed consent
4.	Acknowledgement by the head of the institution of Thiagarajar College Of Preceptors
5.	Acknowledgement by the head of the institution of Senthamil College
6.	Blinding – letter to the Dean of Ultra College of Pharmacy regarding the codes of the intervention
7.	Study proforma
8.	Letter of acceptance of the statistician to help with the statistical analysis of the research
9.	Letter of acceptance of the microbiology lab to help with the microbiological analysis in the research.
10.	Copy of the lab report of the microbiological analysis.

ANNEXURE 1

ETHICAL CLEARANCE FROM INSTITUTIONAL REVIEW BOARD

BEST DENTAL SCIENCE COLLEGE
ULTRA TRUST
69/1A, Ultra Nagar, Madurai - Chennai Highway,
Madurai - 625 104. Ph : 0452 2423290 / 91

Trust Regd.office : 4/235, College Road, Thasildhar Nagar, Madurai - 625020. Ph : 2534593, 2534701 Fax : 91-452-2539828

ANNEXURE-I

Ref:UT:BDSC:IRB-EC/2014 Date:18.11.2014

From

Institutional Review Board-Ethical committee,
Best dental science college,
Madurai .

To

The Controller of Examinations ,
The Tamil Nadu DR.MGR Medical University,
No. 69, Anna salai,
Guindy,
Chennai-600 032

Sir/Madam

The Dissertation topic titled "COMPARATIVE EVALUATION OF EFFICACY OF GREEN TEA AND CHLORHEXIDINE MOUTHRINSE IN REDUCING THE MUTANS STREPTOCOCCI COUNT IN SALIVA - A RANDOMIZED CONTROLLED TRIAL" submitted by DR. PRISCILLA JOYS.N postgraduate student has been approved by Institutional Review Board of Best Dental Science College on 18.11.2014.

DR.K.S PREM KUMAR.M.D.S.,
VICE PRINCIPAL
MEMBER SECRETARY
INSTITUITONAL REVIEW BOARD-ETHICAL COMMITTEE
BEST DENTAL SCIENCE COLLEGE
MADURAI

DR.PURUSHOTHAM MANVI,M.D.S.,
PRINCIPAL
BEST DENTAL SCIENCE COLLEGE
MADURAI

ANNEXURE II

PATIENT INFORMATION LETTER

PARTICIPANT ID: 11X

PATIENT INFORMATION FORM

Participant' s name: MS. G. JEYABRUNDHA.....

I am Dr.PriscillaJoys.N , Postgraduate student from the Department Of Public Health Dentistry Of Best Dental Science College And Hospital. I am conducting a research *to compare the antibacterial efficacy of Green Tea and Chlorhexidine Mouth Rinses*. The research procedure will involve collection of personal details, examination of your teeth and collection of saliva. In the course of my research you will be allocated randomly into one of the 2 groups of mouth rinses. You will be required to rinse with one of the mouth rinse for a stipulated time. You will be expected to be under my supervision for a duration of approximately 2 hours. These rinses are generally safe with little or no side effects when used orally as a rinse for a short while. However I cannot and do not guarantee that you will receive any direct benefits from this research. But with the results of this research we might possibly be able to recommend these herbal mouth rinses as an efficacious and cost-effective mouth rinses for the maintenance of oral hygiene by the society at large.

You will be given complete information about the research and requested to be part of the same. I shall take the time to make myself clear with the explanation of the intention and the steps involved in the study. You do not have to decide immediately about participating in the study. You may decide about participating in the research after talking to anyone with whom you are comfortable, about the research. Hereby I assure you that the details obtained during the course of the study will be kept confidential with regard to individual identity.

If you are not interested to participate in the research or if you feel uncomfortable, you can withdraw or refuse to participate in the research at any time

Name and address of the investigator:

Dr.PriscillaJoys.N

Post graduate student,
Department of public health dentistry,
Best dental science college and hospital,
Madurai

If you experience any grievance during the course of the research, please feel free to call,

Dr.MuthuKaruppaiah.R. M.D.S 94886 14366

ANNEXURE II

INFORMED CONSENT

PARTICIPANT ID : 11X

BEST DENTAL SCIENCE COLLEGE & HOSPITAL,MADURAI
DEPARTMENT OF PUBLIC HEALTH DENTISTRY

STUDY TITLE: Comparative evaluation of efficacy of Green tea and Chlorhexidine mouthrinses in reducing the Streptococcus mutans count in saliva - A Randomized Control Trial

Investigator:	**Guided by:**
Dr.PriscillaJoys.N	Dr.R.MuthuKaruppaiah M.D.S.,
Post graduate student	Reader

INFORMED CONSENT FORM

Name : ~~M~~/Ms G. JEYABRUNDHA	Address : 1503 Vaikkam periyar nagar madurai-19
Gender : ~~Male~~/Female	
Age : 17 Years	Phone number: 9943207247

I have been informed about the purpose, procedure and period of the study, the benefits and the risks in the language that I understand in a comprehensible manner. It has also been assured to me that the data collected during the course of the study will be maintained confidential with regard to individual identity. I hereby give consent voluntarily to participate in the study.

Signature of participant..... G. Jeyabrundha ..... Date: 2 Aug 2016 .....

Name in block letters..... G. JEYABRUNDHA .....

Investigator's Signature..... Priscilla ..... Date: 2 Aug 2016 .....

Name in block letters..... Dr.PRISCILLA JOYS.N .....

Witness's Signature ..... Sheba.N ..... Date: 2 Aug 2016 .....

Name in block letters..... Ms.SHEBA.N .....

ANNEXURE III

ACKNOWLEDGEMENT BY THE HEAD OF THE INSTITUTION OF THIAGARAJAR COLLEGE OF PRECEPTORS

REPORT OF THE RESEARCH

CONDUCTED FROM 24TH TO 28TH MAY 2016

AT THIAGARAJAR COLLEGE OF PRECEPTORS, MADURAI

TO WHOMSOEVER IT MAY CONCERN

The research titled **"Comparative evaluation of the efficacy of Green Tea and Chlorhexidine mouthrinse in reducing the mutans streptococci levels in saliva – A randomised controlled trial."** was conducted by Dr.Priscilla Joys.N. in the premises of Thiagarajar College of Preceptors, Madurai, with the voluntary participation of 28 students 24th to 28th May 2016

DATE: 16·12·16

Signature of the Principal

PRINCIPAL
THIAGARAJAR COLLEGE OF PRECEPTORS
MADURAI-625 009

ANNEXURE IV

ACKNOWLEDGEMENT BY THE HEAD OF THE INSTITUTION OF SENTHAMIL COLLEGE

REPORT OF THE RESEARCH

CONDUCTED FROM 1ST TO 4TH AUGUST 2016

AT SENTHAMIL COLLEGE, MADURAI

TO WHOMSOEVER IT MAY CONCERN

The research titled **"Comparative evaluation of the efficacy of Green Tea and Chlorhexidine mouthrinse in reducing the mutans streptococci levels in saliva – A randomised controlled trial."** was conducted by Dr.Priscilla Joys.N. in the premises of Senthamil College, Madurai, with the voluntary participation of 40 students from 1st To 4th August 2016

DATE: 16.12.2016

16.12.2016
Signature of the Principal

Dr. S. VIJAYAN, M.A.,M.Phil.,Ph.D.,D.G.T.D.J.
PRINCIPAL
SENTHAMIL COLLEGE
MADURAI-625 001

SENTHAMIL COLLEGE PIN 625 001. MADURAI

ANNEXURE V

BLINDING – LETTER TO THE DEAN OF ULTRA COLLEGE OF PHARMACY REGARDING THE CODES OF THE INTERVENTION

DEPARTMENT OF PUBLIC HEALTH DENTISTRY

BEST DENTAL SCIENCE COLLEGE

MADURAI

To

The Dean,
Ultra college of pharmacy,
Madurai

RESEARCH TITLE : Comparative evaluation of efficacy of Green Tea and Chlorhexidine mouthrinses in the reduction of Streptococcus mutans count in saliva – A Randomized Control Trial

GUIDE: Dr.Muthu Karuppaiah.R M.D.S

PRINCIPAL INVESTIGATOR: Dr Priscilla Joys.N II year Postgraduate.

Respected Madam,

On account of **BLINDING**, The following codes are assigned to the interventions and will not be revealed to the principal investigator and the participants until the end of the research.

CODE	INTERVENTION
X	Chlorhexidine
Y	Green Tea

Signature of the Guide

ANNEXURE VI

STUDY PROFORMA – PAGE 1

BEST DENTAL SCIENCE COLLEGE , MADURAI
DEPARTMENT OF PUBLIC HEALTH DENTISTRY
RESEARCH PROFORMA

PARTICIPANT ID:

COMPARATIVE EVALUATION OF EFFICACY OF GREEN TEA AND CHLORHEXIDINE IN REDUCING THE STREPTOCOCCUS MUTANS COUNT IN SALIVA - A RANDOMIZED CONTROL TRIAL

DEMOGRAPHIC DATA

NAME OF SUBJECT:

AGE: Years GENDER: MALE/FEMALE

ADDRESS:

PHONE NUMBER:

EDUCATION

OCCUPATION: INCOME:

SOCIOECONOMIC STATUS:

MEDICAL HISTORY:

ANTIBIOTICS USAGE IN THE LAST 2 WEEKS:

ALLERGIC TO DRUGS/HERBAL CONSTITUENTS:

DENTAL HISTORY:

ORAL HYGIENE PRACTICES AND HABITS:

BRUSHING FREQUENCY:

USE OF FLUORIDE TOOTHPASTE IN THE LAST 48 HRS:

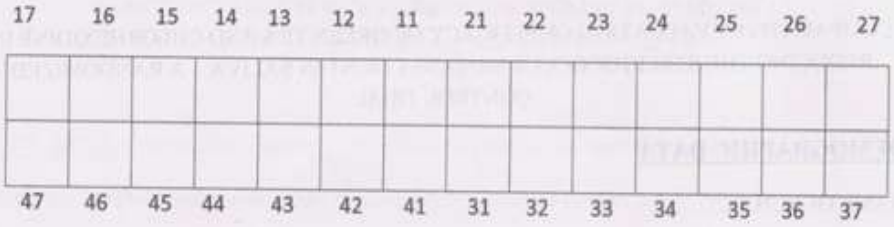
USE OF MOUTHWASHES IN THE LAST 48 HRS:

HABITS:

ANNEXURE VI

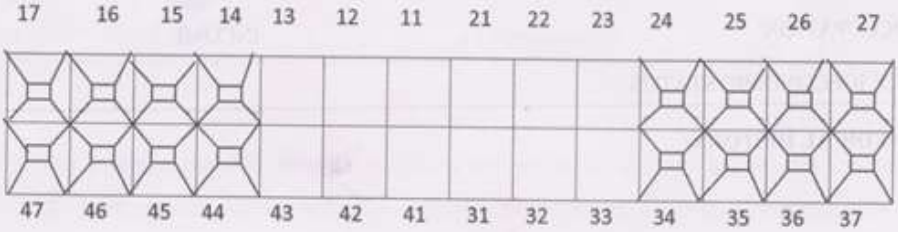
STUDY PROFORMA – PAGE 2

DECAYED, MISSING, AND FILLED TEETH INDEX - (HENRY.T.KLEIN, CARROLE.E.PALMER AND KNUTSON.J.W , 1938)



DT = MT = FT = DMFT =

DECAYED, MISSING, AND FILLED SURFACES INDEX - (HENRY.T.KLEIN, CARROLE.E.PALMER AND KNUTSON.J.W , 1938)



DS = MS = FS = DMFS =

LAB ANALYSIS:

	SALIVARY LEVELS OF MUTANS STREPTOCOCCI (CFU)	pH
PRE-SUCROSE CHALLENGE RINSE:		
POST-SUCROSE CHALLENGE RINSE:		
PRE INTERVENTION AND SUCROSE CHALLENGE RINSE:		
POST INTERVENTION AND SUCROSE CHALLENGE RINSE		

ANNEXURE VII

LETTER OF ACCEPTANCE OF THE STATISTICIAN TO HELP WITH THE STATISTICAL ANALYSIS OF THE RESEARCH

FROM

Dr.Priscilla Joys.N.
Second year postgraduate student,
Department of Public Health Dentistry,
Best Dental Science College And Hospital,
Madurai.

THROUGH

The Head of the Department,
Best Dental Science College and Hospital,
Madurai.

TO

Mr.K.Asaithambi, M.Sc., D.P.D., D.J.M.C.,
(Retd) Lecturer in statistics and Demography,
Research officer,
ICMR, Madurai Medical College,
Madurai

Respected Sir,

SUB: Request to provide help with the statistical analyis in conjunction with the Research work

With reference to the above subject, I would like to bring to your kind notice that I,Dr.Priscilla Joys, Second Year Post Graduate In The Department Of Public Health Dentistry, have planned to conduct a research titled **"Comparative evaluation of the efficacy of Green Tea and Chlorhexidine mothrinse in reducing the mutans streptococci levels in saliva – A randomised controlled trial."** for which I need to do the statistical analyis. Therefore sir, I kindly request you to help me with the statistical analysis. I would be immensely obliged if you could help me with the analysis.

Thanking you,

Date: 15.4.2016

Yours Sincerely,

Priscilla Joys N.

Hereby I express my acceptance to help you with statistical analysis for your research work.

16/4/16

PROFESSOR & H.O.D.,
DEPT.OF PUBLIC HEALTH DENTISTRY,
BEST DENTAL SCIENCE COLLEGE,
MADURAI-625104.

ANNEXURE VIII

LETTER OF ACCEPTANCE OF THE MICROBIOLOGY LAB TO HELP WITH THE MICROBIOLOGICAL ANALYSIS IN THE RESEARCH

FROM

Dr.Priscilla Joys.N.

Second year postgraduate student,

Department of Public Health Dentistry,

Best Dental Science College And Hospital,

Madurai.

THROUGH

The Head of the Department,

Best Dental Science College and Hospital,

Madurai.

TO

The Managing Director,

Oxylab,

Madurai.

Respected Sir,

SUB: Request to provide help with the laboratory analysis in conjunction with the Research work

With reference to the above subject, I would like to bring to your kind notice that I,Dr.Priscilla Joys, Second Year Post Graduate In The Department Of Public Health Dentistry, have planned to conduct a research titled "Comparative Evaluation Of The Efficacy Of Green Tea And Chlorhexidine Mouthrinses On Mutan Streptococci Levels In Saliva - A Randomized Controlled Trial" for which I need to measure the parameters – salivary and plaque Streptococcus mutans Colony Forming Unit counts. Therefore sir, I kindly request you to help me with this lab analysis at your prestigious laboratory. I would be immensely obliged if you could help me with the analysis.

Thanking you,

Date: 6.5.2016

Yours Sincerely,

Priscilla Joys N

PROFESSOR & H.O.D.,
DEPT.OF PUBLIC HEALTH DENTISTRY,
REST DENTAL SCIENCE COL...
MADURAI-625104.

Accepted help

OXY CLINICAL LABORATORY
19, PATTARAIKARA STREET,
GORIPALAYAM.

ANNEXURE IX

LAB REPORT FROM THE MICROBIOLOGICAL LABORATORY

Oxy Clinical Laboratory & Biopsy Centre
Fully Automated with Digital X-Ray
▪ Digital X-Ray ▪ ECG ▪ Biopsy ▪ PFT

19, Pattaraikara Street, Behind North Gate Hotel,
Goripalayam, Madurai - 625 002.
Phone : 4361633, 2521633
Mobile : 9443477366
E-mail : oxylab@gmail.com

To
Dr.PRISCILLA JOYS.N
Post Graduate Student
BEST Dental Science College
MADURAI

PARTICIPANT ID	SAMPLE A *(Baseline) CFU/ml*	SAMPLE B *(After Sucrose) CFU/ml*	SAMPLE C *(Baseline After 1 Hour) CFU/ml*	SAMPLE D *(After Intervention And Sucrose) CFU/ml*
1X	1,00,000	1,00,000	1,00,000	NO GROWTH
3X	1,00,000	1,00,000	1,00,000	NO GROWTH
5X	1,00,000	1,00,000	1,00,000	NO GROWTH
7X	1,00,000	1,00,000	1,00,000	NO GROWTH
9X	1,00,000	1,00,000	1,00,000	NO GROWTH
11X	1,00,000	1,00,000	1,00,000	NO GROWTH
13X	1,00,000	1,00,000	1,00,000	NO GROWTH
15X	59,000	59,000	29,000	NO GROWTH
17X	1,00,000	1,00,000	1,00,000	NO GROWTH
19X	1,00,000	1,00,000	1,00,000	NO GROWTH
21X	1,00,000	1,00,000	1,00,000	NO GROWTH
23X	1,00,000	1,00,000	1,00,000	NO GROWTH
25X	1,00,000	1,00,000	1,00,000	NO GROWTH
27X	-	-	-	-
29X	1,00,000	59,000	59,000	NO GROWTH
31X	1,00,000	1,00,000	59,000	NO GROWTH
33X	1,00,000	59,000	1,00,000	NO GROWTH
35X	1,00,000	1,00,000	1,00,000	NO GROWTH
37X	1,00,000	1,00,000	1,00,000	NO GROWTH
39X	1,00,000	1,00,000	1,00,000	NO GROWTH

OXY CLINICAL LABORATORY
19, PATTARAIKARA STREET,
GORIPALAYAM,
MADURAI-625 002.

LAB REPORT FROM THE MICROBIOLOGICAL LABORATORY

- **CONTD**

Oxy Clinical Laboratory & Biopsy Centre
Fully Automated with Digital X-Ray
▪ Digital X-Ray ▪ ECG ▪ Biopsy ▪ PFT

19, Pattaraikara Street, Behind North Gate Hotel,
Goripalayam, Madurai - 625 002.
Phone : 4361633, 2521633
Mobile : 9443477366
E-mail : oxylab@gmail.com

PARTICIPANT ID	SAMPLE A *(Baseline) CFU/ml*	SAMPLE B *(After Sucrose) CFU/ml*	SAMPLE C *(Baseline After 1 Hour) CFU/ml*	SAMPLE D *(After Intervention And Sucrose) CFU/ml*
2Y	59,000	1,00,000	59,000	29,000
4Y	1,00,000	1,00,000	59,000	29,000
6Y	1,00,000	1,00,000	59,000	59,000
8Y	1,00,000	1,00,000	29,000	59,000
10Y	59,000	29,000	1,00,000	58,000
12Y	1,00,000	1,00,000	1,00,000	29,000
14Y	1,00,000	1,00,000	1,00,000	29,000
16Y	59,000	59,000	59,000	28,000
18Y	1,00,000	59,000	1,00,000	28,000
20Y	59,000	59,000	29,000	29,000
22Y	1,00,000	1,00,000	1,00,000	1,00,000
24Y	1,00,000	1,00,000	1,00,000	1,00,000
26Y	1,00,000	1,00,000	1,00,000	1,00,000
28Y	1,00,000	1,00,000	1,00,000	1,00,000
30Y	1,00,000	1,00,000	1,00,000	1,00,000
32Y	28,000	59,000	59,000	1,00,000
34Y	1,00,000	1,00,000	28,000	28,000
36Y	1,00,000	1,00,000	59,000	59,000
38Y	1,00,000	1,00,000	1,00,000	1,00,000
40Y	1,00,000	1,00,000	1,00,000	59,000

The following are the results of the Mutans Streptococci CFU/ml of the Salivary Samples received from you. They were cultured in Mitis Salvarius Bacitracin Agar, incubated in an anaerobic medium for 48 hours.

OXY CLINICAL LABORATORY
19, PATTARAIKARA STREET,
GORIPALAYAM,
MADURAI-625 002.