TO EVALUATE THE EFFECTS OF RINSING WITH GREEN TEA ON STREPTOCOCCUS MUTANS COUNT IN PLAQUE AND TO COMPARE THE EFFICACY OF GREEN TEA WITH THAT OF CHLORHEXIDINE MOUTH WASH AND PLAIN WATER ON THE STREPTOCOCCUS MUTANS COUNT IN PLAQUE: A RANDOMIZED CONTROLLED TRIAL

DISSERTATION

Submitted to the

BABU BANARASI DAS UNIVERSITY, LUCKNOW, UTTAR PRADESH

In the partial fulfilment of the requirement for the degree

Of

MASTER OF DENTAL SURGERY

In the specialty of

PUBLIC HEALTH DENTISTRY

By

Dr. SHRADDHA MISHRA

Under the Guidance of

Dr. ANURADHA. P

Professor and Head

Department of Public Health Dentistry

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES, LUCKNOW

BATCH : 2019-2022

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"To evaluate the effects of rinsing with Green tea on Streptococcus mutans count in plaque and to compare the efficacy of Green tea with that of chlorhexidine mouth wash and plain water on the Streptococcus mutans count in plaque: A Randomized Controlled Trial "**is a bonafide and genuine research work carried out by me under the guidance of **Dr. Anuradha P**, **Professor and Head** , Department of Public Health Dentistry, Babu Banarasi Das College of Dental Sciences, Babu Banarasi Das University, Lucknow, Uttar Pradesh.

Date:

Place:

Dr. Shraddha Mishra

CERTIFICATE BY THE GUIDE

This is to certify that the Dissertation titled "**To evaluate the effects of rinsing with Green tea on Streptococcus mutans count in plaque and to compare the efficacy of Green tea with that of chlorhexidine mouth wash and plain water on the Streptococcus mutans count in plaque: A Randomized Controlled Trial.**" is a bonafide work done by **Dr.Shraddha Mishra** under my direct supervision and guidance towards the partial fulfillment of the requirement for the Degree Of Master Of Dental Surgery in the subject of Public Health Dentistry**.**

Date:

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CERTIFICATE BY THE CO-GUIDE

This is to certify that the Dissertation titled **"To evaluate the effects of rinsing with Green tea on Streptococcus mutans count in plaque and to compare the efficacy of Green tea with that of chlorhexidine mouth wash and plain water on the Streptococcus mutans count in plaque: A Randomized Controlled Trial "** is a bonafide work done by **Dr.Shraddha Mishra** under my direct supervision and guidance towards the partial fulfillment of the requirement for the Degree Of Master Of Dental Surgery in the subject of Public Health Dentistry**.**

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This is to certify that the Dissertation titled **"To evaluate the effects of rinsing with Green tea on Streptococcus mutans count in plaque and to compare the efficacy of Green tea with that of chlorhexidine mouth wash and plain water on the Streptococcus mutans count in plaque: A Randomized Controlled Trial"** is a bonafide work done by **Dr. Shraddha Mishra** under the direct supervision and guidance of **Dr. Anuradha P.** Professor and Head of Department of Public Health Dentistry, Babu Banarasi Das college Of Dental Sciences, Babu Banarasi Das University, Lucknow.

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ENDORSEMENT BY THE HEAD OF THE INSTITUTION

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I hereby declare that the Babu Banarasi Das University shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic/ research purpose.

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Place:

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-Dr . Shraddha Mishra

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LIST OF ABBREVIATIONS

S.NO	Abbreviations	Full forms
1.	N	Number of subjects
2.	SD	Standard deviation
3.	±	Plus minus
4.	≥	Greater than or equal to
5.	≤	Less than or equal to
6.	S. mutans	Streptococcus mutans
7.	GT	Green tea
8.	CHX	Chlorhexidine
9.	Group A	Green tea group
10.	Group B	Chlorhexidine group
11.	Group C	Plain water group
12.	CFU	Colony Forming Unit
13.	PI	Plaque Index
14.	Ml	Millilitre
15.	%	Percentage
15.	Sq.km	Square Kilometer
16.	Ft	Feet
17.	SPSS	Statistical Package For Social Sciences
18.	ANOVA	Analysis of Variance
19.	T test	Student t test
20.	IEC	Institutional Ethics Committee

Abstract

Abstract :

Introduction : Dental caries is a disease with multiple etiological factors .Plaque harbours different types of bacteria among which Streptococcus mutans in plaque is responsible for caries initiation. Chlorhexidine mouthwash is conventionally used antibacterial agent but it has reported side-effects on its long term use. Herbal mouthwash can be an effective alternative to gold standard chlorhexidine mouth wash on long term use . The study was conducted with the aim to evaluate the effectiveness of green tea mouth-rinse in reducing Streptococcus mutans in plaque and to assess its efficacy in comparison to gold standard chlorhexidine mouth wash and plain water .

Materials and Methods: It was a single centre multiple arm parallel placebo controlled randomized controlled trial . A total of 90 participants were selected for the study and were divided in 3 groups ie Group A(2% Green Tea), Group B(0,2% Chlorhexidine) , Group C(Distilled water) by envelope method. The participants were stratified according to gender to ensure that equal number of males and females participate in the study. The study was conducted in two phases .In Phase 1 Baseline plaque samples were collected before rinsing with mouthwash In 2nd phase plaque samples were collected after 7 days of using the rinse . Baseline plaque samples and plaque samples collected after 7 days of rinsing were subjected to microbial analysis to assess the effect of intervention in reducing Streptococcus mutans count in plaque after using the rinse . Paired –t test was used for within group comparison and One Way ANOVA test was used for between group comparison. A p-value < 0.05 was considered statistically significant .

Results: The mean reduction in Streptococcus mutans count in plaque sample was more in green tea and Chlorhexidine group in comparison to plain water

group and the values were statistically significant($p<0.05$).On comparing mean reduction in Streptococcus mutans count in plaque sample among Chlorhexidine and Green tea group . Chlorhexidine group showed more reduction in Streptococcus mutans count but there was no statistically significant difference between both the groups ($p>0.05$).

Conclusion : Chlorhexidine mouthwash is still the most superior antibacterial agent , but side effects on its long term use cannot be neglected . Green tea mouthwash , a herbal antibacterial agent is equally efficacious and effective as conventional chlorhexidine mouth-wash with pleasant taste and no side –effects .Hence , it can be used as an alternative to conventional chlorhexidine mouth-wash on routine basis as an adjunct to mechanical plaque removal for a longer period of time .

Keywords: Mouthwash , Green Tea , Chlorhexidine , Plain Water , Plaque , Streptococcus Mutans

Introduction

Introduction :

Dental caries is said to be one of the prevalent bacterial infections that has adverse impacts on oral health in the form of various irreversible sequelae . These adverse impact is overpowered by the fact that this disease is ubiquitous , non –contagious and has no effect on organ systems .

Streptococcus mutans is the major etiological agent responsible for initiation of dental caries , a gram negative facultative anaerobic bacteria. [1] Hence reducing the Streptococcus Mutans count in the oral cavity is the best way to reduce the incidence of dental caries .

Plaque is considered as one of the local factor responsible for oral diseases . Research studies have led to the formulation of different plaque control agents. The mode of administration of antimicrobial agent is primarily important in controlling the rise in bacterial count . Studies have suggested that antimicrobial agent that is administered locally has higher efficiency than systemic application of antimicrobial agent. [2] Antimicrobial agents are very common and have widespread use among these chlorhexidine is the gold standard chemical plaque control agent.

Chlorhexidine has a broad antibacterial activity, very low toxicity, and more substantivity .

Chorhexidine is a bis- biguanide that significantly reduces the total number /count of bacteria in the oral cavity . It interferes with the adherence factors of these cariogenic micro-organisms that are present on the tooth surface. [3] But Chlorhexidine when used for a longer time period causes various side effects like staining of tooth, alterations in taste and mucosal erosions and lesions instead of its beneficial antiplaque effects . [3]

Antiplaque agent made of herbal product is the best way to achieve antimicrobial effect with negligible side effects. [4]

Herbal products are considered a part of Indian culture since decades and is used for the treatment of various ailments affecting systemic health and oral health . Green tea is one of the herbal products with medicinal and antioxidant properties which has a positive effect on overall systemic health . [4]

Green tea is made mainly with the leaves of Camelli a sinensis whose leaves undergoes minimal oxidation during the phase of processing to prepare green tea . [5] Green tea is prepared from the unfermented leaves of the plant. It consists of maximum amount of most active ingredients which are the polyphenols than Oolong tea and Black tea which are partially fermented and completely fermented analogues of green tea. [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. Green tea was originated in China at around third century AD. It's first consumption was

to decrease anxiety disorders , depression and gastro-intestinal ailments due to its high medicinal properties . Gradually there was a substantial increase in its popularity and it came under the category of most common and widely used beverage in China. [7]

It also has anticarcinogenic effects and is considered as one of the most efficient and powerful antimicrobial and antiviral agent. Polyphenols is one of the major component in green tea which inhibits the growth of pathogenic bacteria that cause oral and periodontal diseases and thereby directs positive impact and improves oral and gingival health. [8] The polyphenols target the virulence factors of the gram negative Streptococcus mutans and also inhibit the adherence of micro-organisms to the tooth surface [9] thereby decreasing the acid production in the oral cavity and increase the pH of the saliva . These activities confers anticariogenic properties to green tea . [10]

In 1989, it was demonstrated that Japanese Green tea has an inhibitory effect on Streptococcus mutans leading to its subsequent decrease in colony forming unit(CFU) . The results of in-vitro tests stated that the **Minimum Inhibitory Concentration (MIC)** of Green tea polyphenols was **250-1000 microgram/ml**. [11]

Epidemiological studies done in the past are more centered towards evaluation of the systemic health benefits of green tea . Studies done pertaining to its antimicrobial effect on Streptococcus mutans count as mouth rinse and further comparative evaluation of its efficacy with that of chlorhexidine mouth wash are very few .

The aim of the study is to evaluate the effects of rinsing with Green tea on Streptococcus mutans count in plaque .

Aim & objectives

Aim :

To evaluate the effects of rinsing with Green tea on Streptococcus mutans count in plaque.

Objectives :

1. To evaluate effects of rinsing with Green tea on Streptococcus mutans count in plaque
2. To compare the efficacy of Green tea with that of chlorhexidine mouth wash and plain water on the Streptococcus mutans count in plaque

.

Hypothesis of the study :

Null Hypothesis:

There is no statistically significant difference in mean Streptococcus mutans count(CFU/ml) between Group A(2% Green tea) , Group B(0.2% Chlorhexidine gluconate) and Group C(plain water) .

Alternate Hypothesis :

There is statistically significant difference in mean Streptococcus mutans count(CFU/ml) between Group A(2% Green tea) , Group B(0.2% Chlorhexidine gluconate) and Group C(plain water) .

Review of Literature

Review of Literature :

1. **S Sakanaka, Kim M, Taniguchi M, Yamamoto T. [1989]** [11] 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.

2. **Otake S, Makimura M, Kuroki T, Nishihara Y, Hirasawa M. [1991]** [9] 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.

3. **Hirasawa M, Takada K, Otake S. [2006]** [12] 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.

4. **Molinari M, Watt KD, Kruszyna T, Nelson R, Walsh M, Huang WY, Nashan B, Peltekian K .[2006]** [13] conducted a case report and review

study on Acute liver failure induced by green tea extracts in which they 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.

5. **Chacko SM, Thambi PT, Kuttan R, Nishigaki I. [2013]** [14] conducted a review study on Beneficial effects of green tea 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.

6. **Naderi NJ, Niakan M, Kharazi Fard MJ, Zardi S.[2011] [8]** conducted a study on antibacterial activity of Iranian green and black tea on streptococcus mutans 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.

7. **Ferrazzano GF, Roberto L , Amato I, Cantile T, Sangianantoni G , Ingenito A. [2011][15]** 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.

8. **Awadalla et al [2011] [10]** l9 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.

9. **Subramaniam P, U Eswara, KM Reddy .[2011][16]** 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 . Epicatechin , Epigallocatechin 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.

10. **Moezizadeh M et al [2012][17]** 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 Review of Literature 10 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.

11. **Balappanavar** A **, Sardana V , Singh [2013]** [18] conducted a randomized control trialto evaluate and compare the effectiveness of 0.5% tea, 2% neem, and 0.2% chlorhexidine mouthwashes on oral health(2013). 30 healthy human volunteers of age group 18-25 years was carried out. The subjects were randomly assigned to 3 groups i.e., group A - 0.2% chlorhexidine gluconate (bench mark control), Group B - 2% neem, and group C - 0.5% tea of 10 subjects per group. Plaque accumulation and gingival condition were recorded using plaque index and gingival index. Oral hygiene was assessed by simplified oral hygiene index (OHIS). Salivary pH was assessed by indikrom pH strips. Plaque, gingival, and simplified OHI scores as well as salivary pH were recorded at baseline, immediately after 1 st rinse, after 1 week, 2 nd week, and 3 rd week. The 3 rd week was skipped for group A. Mean plaque and gingival scores were reduced over the 3 week trial period for experimental and control groups. Anti-plaque effectiveness was observed

in all groups and the highest being in group C ($P < 0.05$). Neem and tea showed comparative effectiveness on gingiva better than chlorhexidine ($P < 0.05$). The salivary pH rise was sustained and significant in Group B and C compared to Group A. Oral hygiene improvement was better appreciated in Group B and Group C. The effectiveness of 0.5% tea was more compared to 2% neem and 0.2% chlorhexidine mouth rinse.

12. **RS Neturi, R Srinivas , B Vikram Simha , Y Sree, Sandhya Chandra , T Shekar, S Kumar [2014]** [19] 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.

13. **Kaur H , Jain S [2014]**[4] conducted a randomized control trial to compare the antiplaque efficacy of green tea catechin mouthwash with chlorhexidinegluconatemouthwashA single blinded cross-over study was conducted among 30 participants in the age group of 18-25 years. The mouthwash samples for the study were previously labeled assigning the letters: A (0.25% of green tea catechin mouthwash) and B (0.12% of

chlorhexidine mouthwash). The study subjects were randomly divided into two groups of 15 each and the study was divided into two phases. In phase I, the mouthwash A was given to one group and other group was given mouthwash B. After a 15 day washout period, in phase II, both groups were given otThe plaque scores were compared and the difference between the green tea catechin and chlorhexidine mouthwash was determined by t-test. The difference between plaque scores were not statistically significant (P > 0.05). The results showed that both the groups that is green tea catechin mouthwash (0.25%) and chlorhexidine mouthwash (0.12%) have comparable results in plaque reduction. her mouthwash. At the end of each phase of 1 week, plaque score was recorded by using Turesky modification of the Quigley-Hein plaque index. The plaque scores were compared and the difference between the green tea catechin and chlorhexidine mouthwash was determined by t-test. The difference between plaque scores were not statistically significant (P > 0.05). The results showed that both the groups that is green tea catechin mouthwash (0.25%) and chlorhexidine mouthwash (0.12%) have comparable results in plaque reduction. This study supports the effectiveness of green tea catechin mouthwash as an antiplaque agent. It should be explored as a cost-effective, long-term antiplaque rinse with prophylactic benefits.

14. **Rao.A et al [2014]**[20] 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 370 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.

15. **Fajriani F, Andriani JN [2014]**[21] 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.

16.. **Thomas A, Thakur S, Mhambrey S.[2014]**[22] 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.

17. **Abdelmegid F, Al-Agamy M, Alwohaibi A, Ka'abi H, Salama F[2015]**[23] (conducted a study on ross-sectional in vivo study was to assess the effect of green tea and honey solutions on the level of salivary Streptococcus mutans. Study design: A convenient sample of 30 Saudi boys aged 7–10 years were randomly assigned into 2 groups of 15 each. Saliva sample was collected for analysis of level of S. mutans before rinsing. Commercial honey and green tea were prepared for use and each child was asked to rinse for two minutes using 10 mL of the prepared honey or green tea solutions according to their group. Saliva samples were collected again after rinsing. The collected saliva samples were prepared and colony forming unit (CFU) of S. mutans per mL of saliva was calculated. Results: The mean number of S. mutans before and after rinsing with honey and green tea solutions were $2.28*10^8$($2.622*10^8$), $5.64*10^7$($1.03*10^8$), $1.17*10^9$($2.012*10^9$) and $2.59*10^8$ ($3.668*10^8$) respectively. A statistically significant reduction in the average number of S. mutans at baseline and post intervention in the children who were assigned to the honey (P=0.001) and green tea (P=0.001) groups was found. Conclusions: A single time mouth rinsing with honey and green tea solutions for two minutes effectively reduced the number of salivary S. mutans of 7–10 years old boys.

18. **Cardoso KB, Carvalho MD, Kudo GA, Matsumoto MA, Junior S, Ferreira J, Saraiva PP.[2016]**[24] 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.

19. **Nandan N , Prasanna M , Prem Kishore K. [2016]** [25] conducted a study on Effect of Green Tea as a Mouth Rinse on Streptococcus mutans . A significant reduction in plaque S.mutans counts was observed with Green tea mouth rinse, which was not significantly different from that of the time tested chlorhexidine mouth rinse. The study concluded that the use of a green tea as a mouth rinse appears to be effective for regular use as part of a daily preventive regime in children.

20. **Singh O, Reddy VK, Pradhan D, Sharma L et al [2019]** [26] conducted a study to compare and evaluate the effects of commercially available green tea mouthwash with chlorhexidine mouthwash on gingivitis .A 3-week, double-blinded study was conducted on thirty nursing students aged between 18 and 25 years. Plaque status was assessed by plaque index (Silness and Loe 1964) and gingival status was assessed by gingival index (Loe and Silness 1963) at the 14^{th} day and at the 21^{st} da. The study concluded that green tea mouthwash was found to be more effective than the chlorhexidine mouthwash in reducing extensive levels of gingivitis.

Materials & Methods

`Materials and Methods :

The present study was done to evaluate the effects of rinsing with Green tea on Streptococcus mutans count in plaque.

Study Area :

The study was conducted in the Department of Public Health Dentistry , BBDCODS BBDU, Lucknow city, Uttar Pradesh in collaboration with Department of Microbiology , Ram Manohar Lohiya Institute of Medical Sciences, Lucknow for microbial analysis. It is the capital city of the Indian state of Uttar Pradesh .

The city is situated at an elevation of 123 metres (404 ft) above sea level.

Lucknow district covers an area of 2,528 square kilometers (976 sqm) .

It is surrounded on Eastern side by district Barbanki , on the western side by district Unnao , on the southern side by Raebarelli and on the northern side by Sitapur and Hardoi.

According to provisional Census report of India 2011 , Lucknow city had a population of 2,815,601, of which 1,470,133 were men and 1,345,468 women. This was an increase of 25.36% compared to the 2001 Census report of India.

The city has a total literacy level in 2011 of 84.72% compared to 67.68% for Uttar Pradesh as a whole.

Study Design :

The study is a, parallel multiple arm , in vivo comparative experimental trial designed to assess the effect of rinsing with Green tea on Streptococcus mutans count in plaque.

Study Population :

A randomized controlled trial was conducted on students of 18-25 years of age studying at Babu Banarasi Das University.

Sampling Technique :

Simple random sampling technique was used to select the subjects for the study .

Eligibility Criteria :

Inclusion Criteria

- Both genders.
- Participants of 18-25 years of age
- Participants without known systemic illness.

- Participants with plaque score of 1 according to Silness and Loe Plaque Index .

Exclusion Criteria

- Participants with known systemic illness.
- Subjects who are on medication.
- Participants with history of drug use for at least 3 months prior to the study .
- Participants included in the pilot study.
- Undergoing any dental treatment
- Subjects with no history of use of any other mouthwash or use of green tea and any other medicated prophylactic dentifrices in any form for at least 3 months prior to the study .
- Visit to dentist within 6month.
- Subjects not willing to participate in the study.

Pilot Study :

Pilot study was conducted in Babu Banarasi Das College of Dental Sciences ,BBDU, Lucknow in the month of December 2020 . A total of 24 participants

8 in each group were recruited for the pilot study to check for the feasibility of the study and subjects included in the pilot study were excluded from the main study. Practical issues like the acceptability of the mouthrinses by the subjects, plaque collection, the time taken to transport the plaque samples to the lab, microbiological analyses of the plaque samples were all tested during this pilot study. Appropriate adjustments were made in the methodology and implemented in the main study.

Sample size estimation :

The sample size for the present study was determined scientifically. The data required for determining the sample size was obtained from a previous literature. **[19] [26]**

Sample size was calculated using G-power analysis software . Power of the study was taken as 80% , alpha error as 5% , effect size as 0.25, beta error as 20% and confidence interval at 95% a sample of 28 was obtained for each group . Making an allowance of 10% for attrition, the required sample size for each group is 30. There are 3 groups hence taking 30 subjects in each group 90 healthy volunteers were involved in the present study.

Calibration and Training :

Prior to the study , investigator [1] was trained and calibrated in the Department of Public Health Dentistry, Babu Banarasi Das College of Dental Sciences . Training of the investigator was carried out on the out-patients under the guidance of a trained person. About eight subjects were examined to assess the consistency of intra-examiner reproducibility and intra-examiner reliability . The agreement for most assessments was expected to be 90%.

Selection of Study participants :

Clinical Examination :

The study was conducted in the Department of Public Health Dentistry, Babu Banarasi Das College of Dental Sciences ,BBDU, Lucknow.

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.

Subjects examined were seated in a ordinary chair with a head supported by wall with the examiner standing by the side of the chair.

A study specific proforma was used to record and evaluate demographic details ,medical and dental history of each participant.

The plaque score was calculated through Silness and Plaque index given by Silness J & Loe H in the year 1964 and subjects with (Plaque score =1) were included in the study.

Out of 300 screened students ,130 fulfilled the eligibility criteria . From the 130 eligible study particpants , final required sample size of 90 subjects were randomly selected .

Recording clerk :

Recording clerk was assigned to record the data of study participants. The Recording clerk was well versed pertaining to terms and codes of the survey prior to survey to avoid any error while recording data of study participants.

Survey forms :

Appropriate number of Study specific specially designed proforma was used during the study

Trial design :

Allocation Ratio : 1:1:1

Allocation Sequence :

Participants were allocated to groups on the basis of envelope method.

A box containing concealed envelopes was used for this purpose. Each envelope carried either code “A” . “B” or code “C”.

The enrollment was stratified by gender to ensure that equal number of males and females were allocated to each group (n=15 males and 15 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.

Type of randomization :

Simple Randomization was the type of randomization used for the study.

Unit of randomization:

Plaque Score=1 was used as unit of randomization

Blinding:

The study was a triple blind clinical trial .The study subjects remained blind to the type of mouthrinse they were given. The investigator , microbiologist and

statistician remained blinded to the mouthrinse which were given to the study participants .To ensure this the interventions were kept in brown colored opaque containers. They were each labeled as either code “A”, “B” or “C”. The codes were broken only after the clinical trial was completed.

Implementation :

Participants were assigned to the group and generation of sequence was done by the organizing clerk.

Ethical Issues :

The nature and purpose of the study was explained to the Institutional Review Board and ethical clearance was obtained and permission to conduct the pilot study and the main study in the colleges was obtained by the Institutional Ethical Committee of Babu Banarasi Das College of Dental Sciences , Babu Banarasi Das University , Lucknow ,Uttar Pradesh.

Consent :

The purpose of the study was explained in detail using simple and comprehensible language with the help of the patient information letter. Informed consent was obtained from all the research participants.

Schedule of the survey :

The study was done from August 2021 - September 2021

Intervention :

Preparation of 2% Green Tea:

Fresh green tea (packing date less than one month) was procured from local market which is available in the form of green tea dip bags(LIPTON GREEN TEA , Manufacturer: Lipton, Country of Origin : India)

Two percent green tea was prepared with 2 grams of green tea dip bag dipped in 100 ml hot water for five minutes.

0.2% Chlorhexidine Gluconate Mouthwash :

Commercially available 0.2% Chlorhexidine gluconate mouthwash (Hexidine , ICPA Health Products Ltd India)) was used as positive control . Chlorhexidine was chosen as it is hailed as the "gold standard" mouthwash. 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. Previous researches have documented the initial suppression of Streptococcus mutans in the plaque after administration of chlorhexidine.

Plain water :

Plain water was used as placebo or negative control

Materials and Equipments :

For clinical examination :

Examination gloves

Mouth mask

Head cap

Shoe cover

Surgical gown

Face Shield

Patient Drape

Plain Mouth mirrors

Shepherd hook probes

Tweezers

Sterilized cotton and gauze pieces

Cotton holder

Disposable glasses

Antiseptic solution

Kidney trays

Sterile cotton buds

Disclosing Solution(Two Tone Disclosing solution -DPI Alphaplac)

Disposable cups of 10 ml each

For microbiological study

2% green tea mouthwash

0.2% of chlorhexidine gluconate mouth wash(Hexidine , ICPA Health Products Ltd India)

Plain water

Inoculation loop

Incubator

Sterile test tube containing thioglycolate transport media.

Culture medias (Blood Agar plates)

MOUTH MASK

EXAMINATION GLOVES

HEAD CAP

SHOE COVER

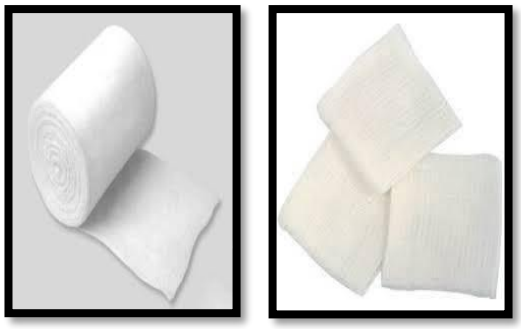
FACE SHIELD

SURGICAL GOWN

PATIENT DRAPE

DIAGNOSTIC INSTRUMENTS

FIGURE 1: MATERIALS AND EQUIPMENTS USED IN THE STUDY (FOR CLINICAL EXAMINATION)



STERILIZED COTTON & GAUZE PIECES

COTTON HOLDER

DISPOSABLE GLASS

HAND SANITIZER , SURFACE DISINFECTANT SPRAY & ANTISEPTIC SOLUTION

FIGURE 2 : MATERIALS AND EQUIPMENTS USED IN THE STUDY (FOR CLINICAL EXAMINATION)

KIDNEY TRAY

STERILE COTTON BUD

DISCLOSING SOLUTION

(TWO TONE DISCLOSING SOLUTION

-DPI ALPHAPLAC)

DISPOSABLE CUPS OF 10 ML

FIGURE 3 : MATERIALS AND EQUIPMENTS USED IN THE STUDY

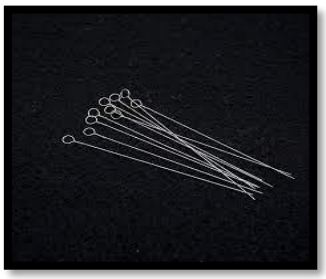
(FOR CLINICAL EXAMINATION)

LIPTON GREENTEA BAGS

0.2 % CHLORHEXIDINE GLUCONATE

THIOGLYCOLLATE TRANSPORT MEDIUM

CULTURE PLATES



INOCULATION LOOP

INCUBATOR

FIGURE 4 : MATERIALS AND INSTRUMENTS USED IN THE STUDY

(FOR MICROBIOLOGICAL STUDY)

STERILITY CONTROL:

Sufficient numbers of instruments were carried to the site of the clinical examination and trial to avoid any interruption in the examinations. The used ones were washed and drained well before sterilization. Disposable gloves and masks were used. A fresh sterile cotton buds were used for study participants and were disposed after every use. The wastes generated during the examination and trial was disposed appropriately.

Methodology:

The study is a single center parallel placebo controlled experimental trial .After obtaining informed consent from the study participants , eligible participants fulfilling the inclusion criteria were allocated one among the 4 groups(via envelope method).The selected 90 participants were divided into four groups:30*3)

Group A: 2% Green tea(Treatment group)

Group B: 0.2% Chlorhexidine gluconate

Group C: Plain water

All the study participants were given 10 ml of three different mouth rinses depending upon the group assigned to them (via enveloped method).All the subjects were instructed to rinse with the given mouth rinses for 30 seconds

twice daily for 7 days after 30 minutes of brushing . The study participants were refrained from using any other medicated prophylactic tooth paste , interdental cleaning aids and other mouthrinses except the intervention(three different mouth rinses) during the study phase and were instructed to avoid drinking tea , coffee 1 hour after using the mouthwash. The subjects were reminded at regular intervals about the instructions through phone calls.

The study was done in two phases.

First Phase :

In first phase plaque sample of all the 90 study participants were collected from lingual surface of lower molars using sterile cotton bud before they were subjected to three different interventions .

The collected plaque sample was transferred to sterile test tube containing transport media (thioglycolate broth) and was send to the laboratory for microbial analysis within half an hour of sample collection .This was done to obtain the base line analysis .

Second Phase:

In second phase all the 90 subjects were instructed to rinse with the 10 ml of given mouth rinses for 30 seconds twice daily for 7 days . After 7 days they were recalled and plaque sample was collected in the same manner as

described in first phase and was send to the laboratory for microbial analysis within half an hour of sample collection.

Microbial analysis :

The plaque samples collected from subjects assigned to three different groups that is Group A (2% green tea), Group B(0.2% chlorhexidine) and Group C(Plain water) were diluted with sterile saline in a ratio of 1:1000 . The plaque samples were streaked on blood agar plates .The plates were incubated for 48 hours at 37 degree Celsius and bacterial colony count was done as Colony forming Unit (CFU/ml).Total Colony count was calculated manually using the formulae :

Total Colony forming unit/ml=total colony count * 1000.

Further the colony count was subjected to statistical analyses to observe the efficacy of Group A (2% green tea) mouthrinse on Streptococcus mutans count and further to compare its efficacy with gold standard mouthrinse or positive control Group B (0.2% chlorhexidine) mouthrinse and placebo or negative control Group C (Plain water) rinse .

Data analysis :-

Data was entered in Microsoft Excel and the analysis was done using SPSS Software version 20(IBM Corporation). Paired t test was used for intragroup comparison between baseline and test values and analysis of variance

(ANOVA) was used for multiple intergroup comparison . Level of significance (p value <0.05) was considered as statistically significant for all of the above mentioned statistical tests.

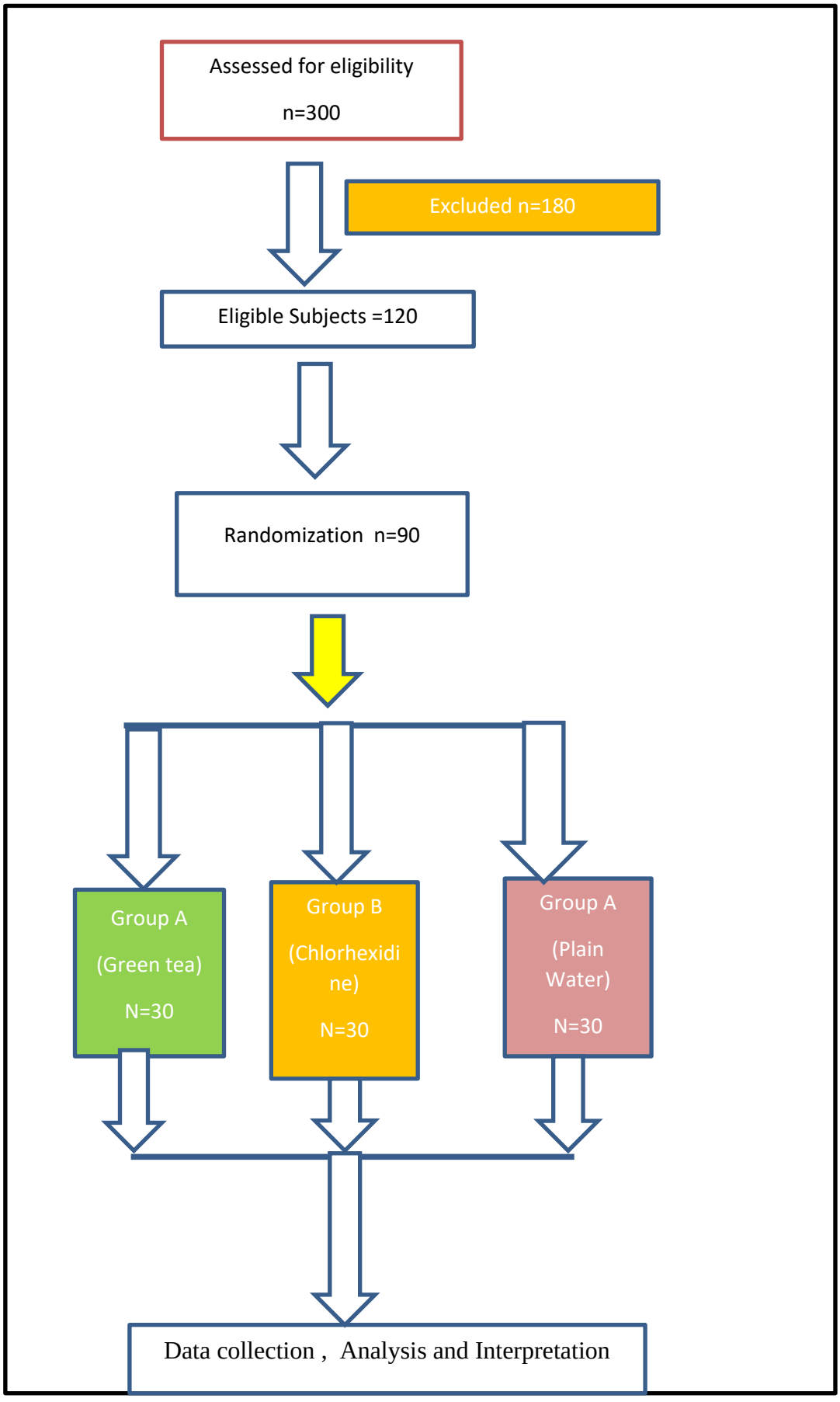
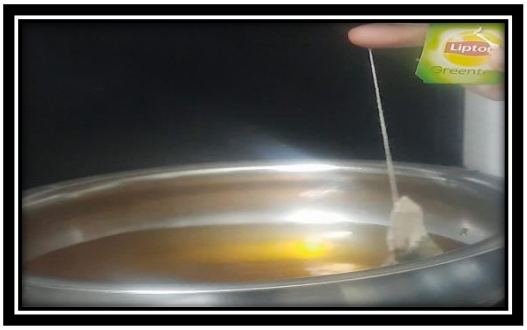


FIGURE 5: SCHEMATIC REPRESENTATION OF METHODOLOGY

FIGURE 6 : BASELINE CLINICAL EXAMINATION

FIGURE 7 : COLLECTION OF PLAQUE SAMPLE USING STERILE COTTON BUD



2 gram Green tea dip bags immersed in 100 ml of hot boiling water

Prepared 2% green tea mouth rinse placed in opaque bottle

FIGURE 8 : PREPARATION OF GREEN TEA MOUTHRINSE

FIGURE 9 : CULTURE PLATES IN THE INCUBATOR

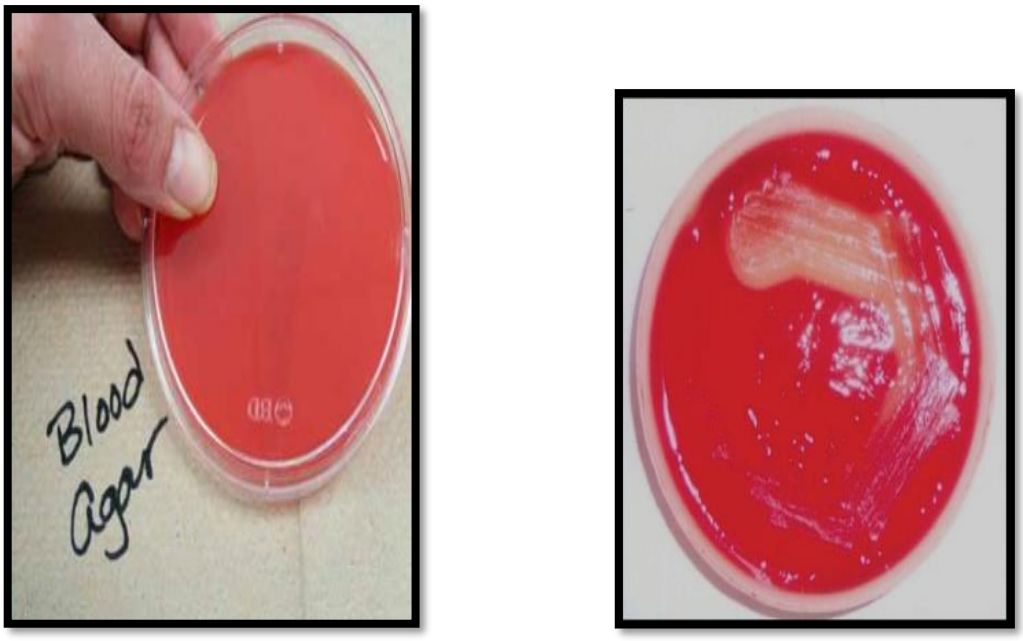


FIGURE 10 : STREPTOCOCCUS MUTANS COLONY IN BLOOD AGAR PLATES

Results

Results :

Table 1a and Graph 1a , Table 1b and Graph 1b shows demographic details of the study participants .

Table 1a and Graph 1a shows Age wise distribution of study participants in three groups (Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) , Group C (Plain Water) .

In Group A(2% Green Tea) , 11(36.7%) study participants were less than or <=20 years of age and 19(63.3%) participants were >20 years of age . Maximum participants were >20 years of age . Mean age group of the study participants in Group A was 21.3± 2.0 years .

In Group B(0.2% Chlorhexidine) , 17(56.7%) study participants were less than or <=20 years of age and 13(43.3%) participants were >20 years of age . Maximum participants were < =20 years of age . Mean age group of the study participants in Group B was 20.7.± 2.1years .

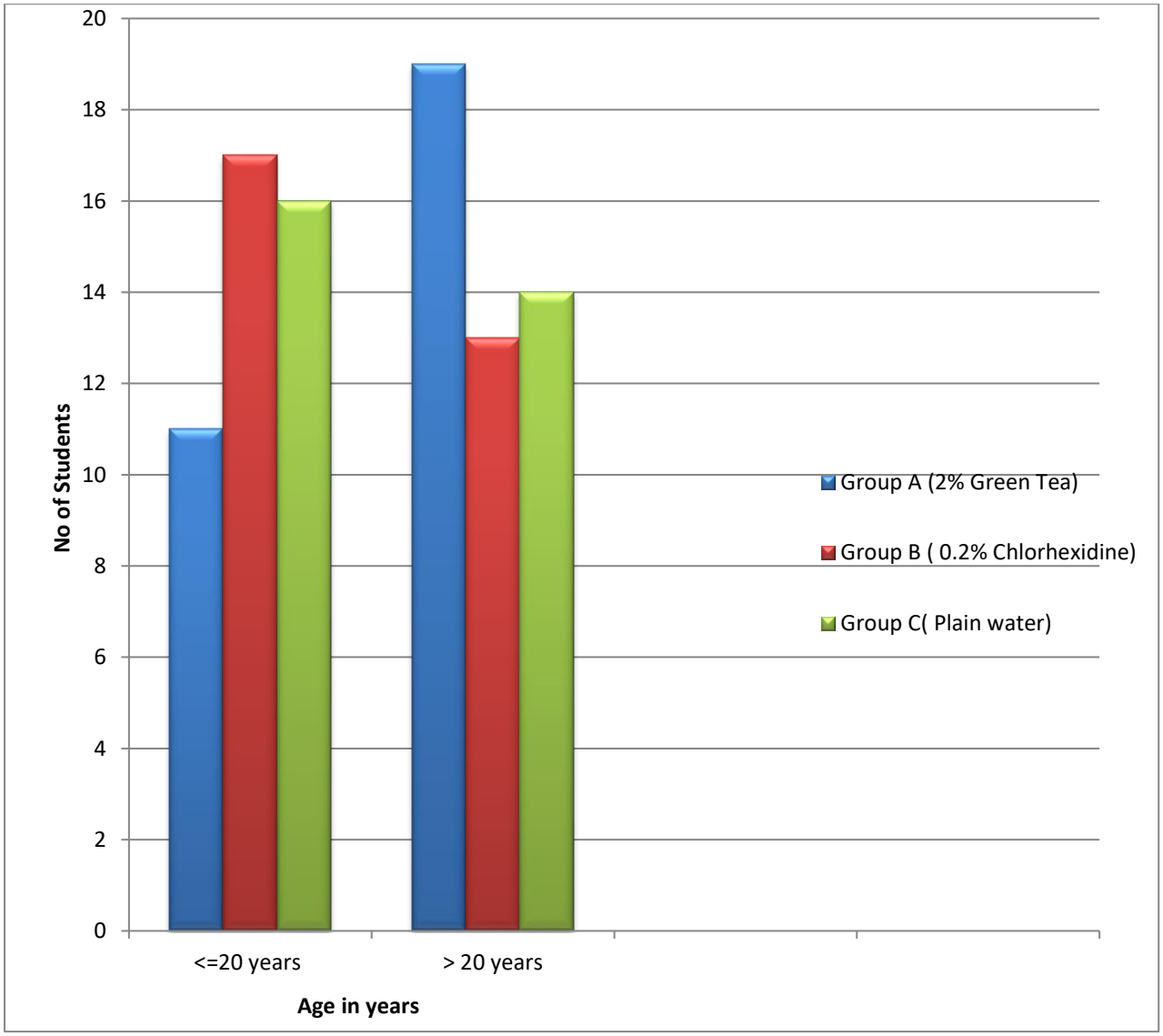
In Group C(Plain Water) , 16(53.3%) study participants were less than or <=20 years of age and 14(46.7%) participants were >20 years of age . Maximum participants were < =20 years of age . Mean age group of the study participants in Group B was 20.6.± 1.8years . The 'p' value was not significant ($p>0.05$)

Demographic details of the study Participants

Age	Group A (2% Green Tea)		Group B (0.2% Chlorhexidine)		Group C (Plain Water)	
	No	**%**	**No**	**%**	**No**	**%**
<=20 years	11	36.7	17	56.7	16	53.3
>20 years	19	63.3	13	43.3	14	46.7
Total	30	100	30	100	30	100
Mean Age	21.3 years		20.7 years		20.6 years	
Standard Deviation	2.0 years		2.1 years		1.8 years	
**** p-value**	0.251					

*Chi -Square test ** p-value<0.05 is statistically significant

Table 1 a : Age wise distribution of study participants in three groups



Graph 1a: Age wise distribution of study participants in three groups

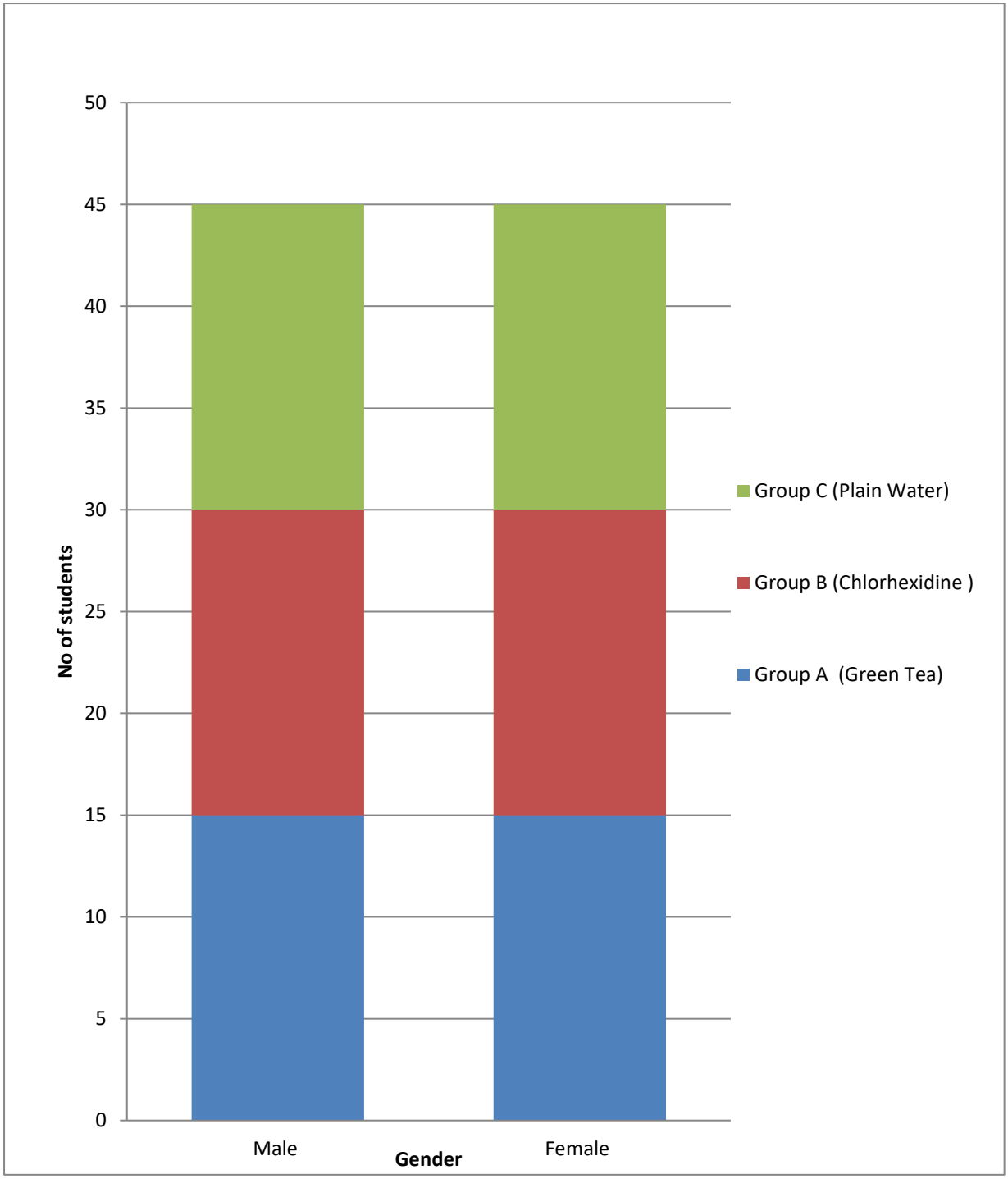
Table 1b and Graph 1b shows gender wise distribution of study participants in three groups (Group A(0.2% Green tea) , Group B(2% Chlorhexidine) , Group C(Plain Water).

A total of 90 subjects were involved in the study . In all the three groups 15(50%) males and 15 (50%) females were involved .The values were not statistically significant (p -value>0.05) . All the 90 subjects participated till the study was conducted depicting no loss to follow-up or attrition.

Gender	Group A (Green Tea)		Group B (Chlorhexidine)		Group C (Plain Water)	
	No	%	No	%	No	%
Male	15	50	15	50	15	50
Female	15	50	15	50	15	50
Total	30	100	30	100	30	100
** p-value	>0.05					

***Chi -Square test ** p-value<0.05 is statistically significant**

Table 1 b: Gender wise distribution of study participants in three groups



Graph 1b : Gender wise distribution of study participants in three groups

Table 2 and Graph 2 shows Baseline *Mean Streptococcus Count* in Group A (2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C (Plain Water) before intervention (Phase 1(before rinse) .

In Group A (2% Green Tea) Baseline Mean *Streptococcus mutans* Count(CFU/ml) before rinsing with 2% Green tea was 32.07±1.83

In Group B (0.2% Chlorhexidine) Baseline Mean Streptococcus mutans Count(CFU/ml) before rinsing with 0.2% Chlorhexidine was 32.25±1.87

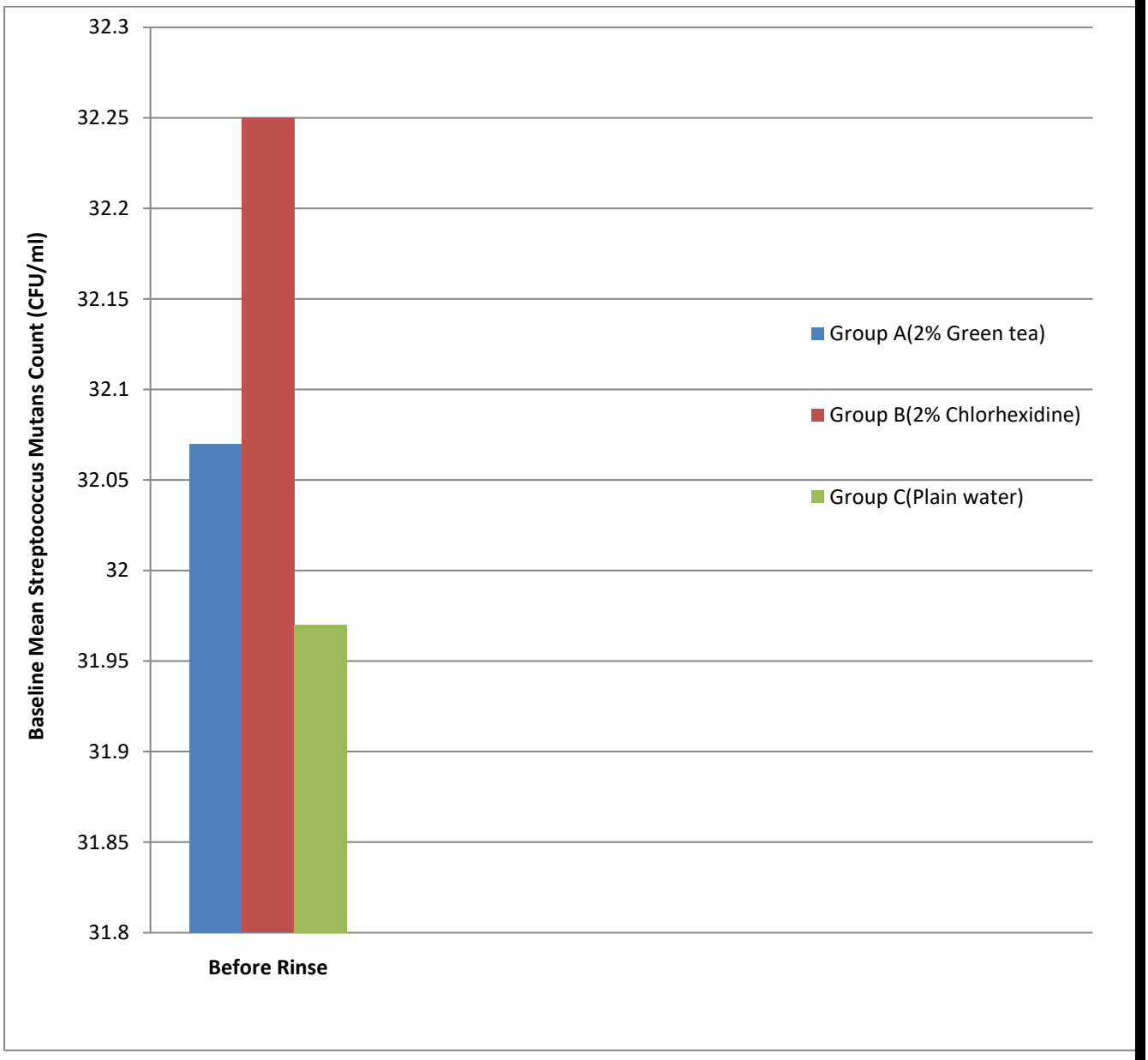
In Group C(Plain Water) Baseline Mean *Streptococcus mutans* Count(CFU/ml) before rinsing with Plain Water was 31.97±1.95

.

Groups	Baseline *Mean Streptococcus count
	Mean ± Standard Deviation
Group A **(2% Green Tea)**	32.07±1.83
Group B **(0.2% Chlorhexidine)**	32.25±1.87
Group C **(Plain Water)**	31.97±1.95

**Mean Streptococcus Count = Colony Forming Unit/ml=Total Colony Count *10^3*

Table 2: Baseline Mean *Streptococcus mutans* count in Group A (2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C (Plain Water) before intervention Phase 1(before rinse)



Graph 2: Baseline Mean Streptococcus mutans count in Group A (2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C (Plain Water) before intervention (Phase 1(before rinse)

Table 3 and Graph 3 shows Mean Streptococcus Mutans count in Group A (2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C (Plain Water) after intervention (Phase 2(after 7 days of rinse)

In Group A (2% Green Tea) Mean Streptococcus Mutans Count(CFU/ml) after 7 days of rinse with 2% Green Tea was 24.54±1.92

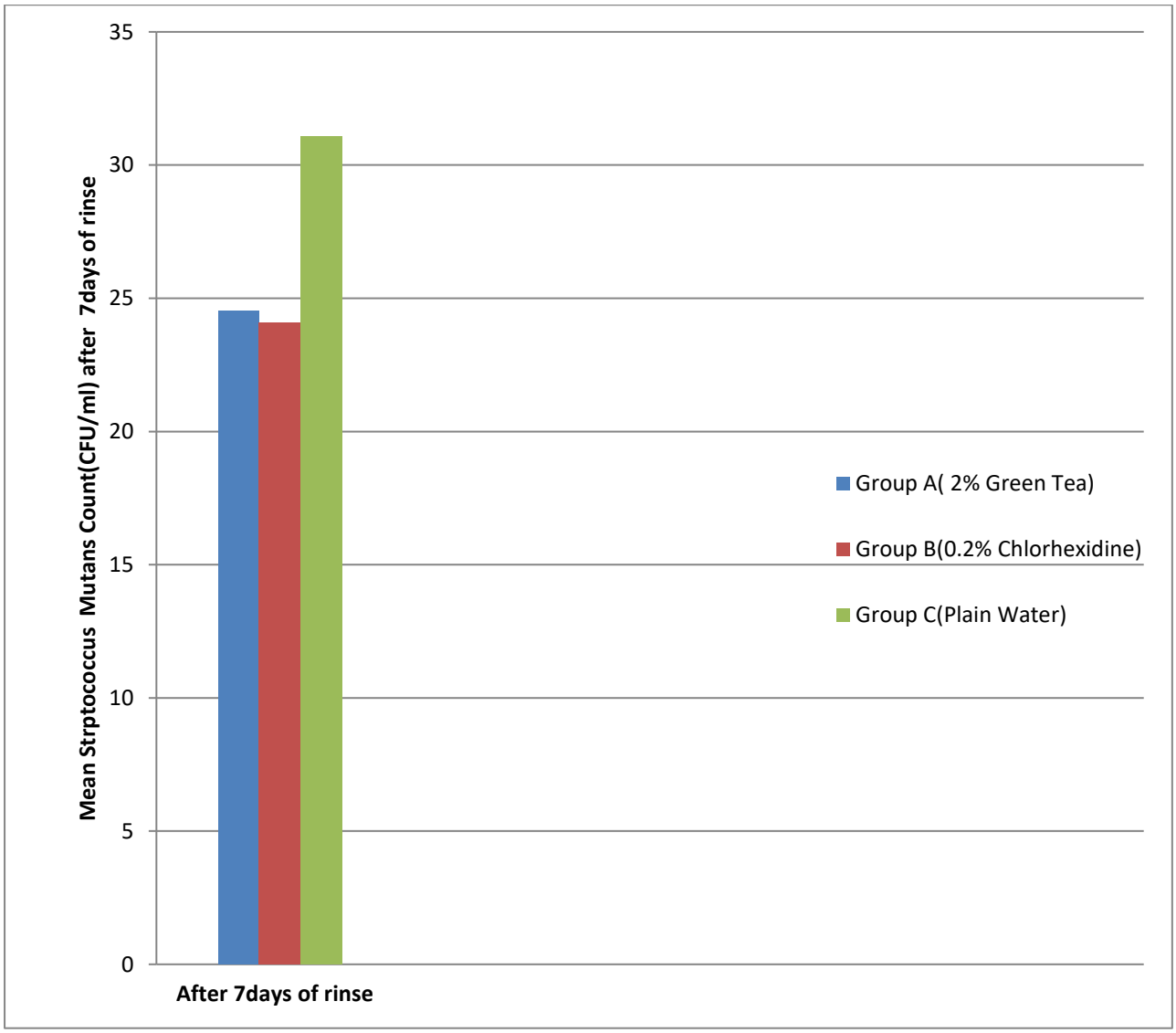
In Group B (0.2% Chlorhexidine) Mean Streptococcus Mutans Count(CFU/ml) after 7 days of rinse with 0.2% Chlorhexidine was 24.09± 1.94.

In Group B (0.2% Chlorhexidine) Mean Streptococcus Mutans Count(CFU/ml) after 7 days of rinse with 0.2% Chlorhexidine was 31.08± 1.63.

Groups	*Mean Streptococcus count (After intervention *(after 7 days of rinse)* Mean ± Standard Deviation
Group A **(2% Green Tea)**	24.54±1.92
Group B **(0.2% Chlorhexidine)**	24.09± 1.94
Group C **(Plain Water)**	31.08± 1.63.

**Mean Streptococcus Count = Colony Forming Unit/ml=Total Colony Count *10^3*

Table 3: Mean Streptococcus mutans count in Group A (2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C (Plain Water) after intervention (Phase 2 (after 7 days of rinse)



Graph 3: Mean Streptococcus mutans count in Group A (2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C (Plain Water) after intervention (Phase 2(after 7 days of rinse)

Table 4 shows Intragroup comparison of Mean *Streptococcus Mutans* Count (CFU/ml) among Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water) over two phases(Phase 1(Before Rinse)) , Phase 2 (7 days after rinse).

In Group A(2% Green Tea) before rinsing with 2 % Green Tea Mean *Streptococcus Mutans* count (CFU/ml) was 32.07±1.83 and after 7 days of rinsing it was 24.54±1.92. There was a statistically significant reduction in the Mean Streptococcus mutans count before and after 7 days of rinse ($p<0.05$).

In Group B(0.2%Chlorhexidine) before rinsing with 0.2% Chlorhexidine mean streptococcus mutans count (CFU/ml) was 32.25±1.87 and after 7 days of rinsing it was 24.09± 1.94. There was a statistically significant reduction in the Mean Streptococcus mutans count before and after 7 days of rinse with 0.2% Chlorhexidine ($p<0.05$).

In Group C (Plain Water) before rinsing with Plain Water Mean Streptococcus Mutans count (CFU/ml) was 31.97±1.95 and after 7 days of rinsing it was 31.08± 1.63. There was no statistically significant reduction in the mean streptococcus count before and after 7 days of rinse with plain water ($p>0.05$)

Phases	Group A (2% Green Tea)		Group B (0.2% Chlorhexidine)		Group C (Plain Water)	
	# Streptococcus Mutans Count (CFU/ml)		# Streptococcus Mutans Count (CFU/ml)		#Streptococcus Mutans Count (CFU/ml)	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Phase 1(Before Rinse)	32.07	1.83	32.25	1.87	31.97	1.95
Phase 2 (7 days after rinse)	24.54	1.92	24.09	1.94	31.08	1.63
***t**	19.17		16.35		1.83	
****p-value**	<0.05		<0.05		>0.05	

*Paired - t test

**p-value<0.05 is Statistically Significant

*Mean Streptococcus mutans count = Colony Forming Unit/ml=Total Colony Count *10^3*

Table 4: Intragroup comparison of Mean Streptococcus mutans count (CFU/ml) among Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water) over two phases(Phase 1(Before Rinse)) , Phase 2 (7 days after rinse)

Table 5a shows Intergroup comparison of Mean Streptococcus Mutans count (CFU/ml) among Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water) over two phases(Phase 1(Before Rinse)) , Phase 2 (7 days after rinse) .

The mean Streptococcus Mutans Count (CFU/ml) before 7 days of rinse in Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water) was 32.07±1.83 , 32.25±1.87 and 31.97±1.95 respectively . There was no statistically significant difference before rinse in Mean Streptococcus Count among Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water) (p value<0.05).

The mean reduction in the Streptococcus mutans (CFU/ml) after 7 days of rinse in Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water) was 24.54±1.92 , 24.09± 1.94 and 31.08±1.63 respectively. There was statistically significant reduction in mean streptococcus count (CFU/ml) after 7 days of rinse among Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C (Plain Water) (p value <0.05).It was more in Group B(0.2% Chlorhexidine) than Group A(2% Green Tea) and it was least in Group C (Plain Water).

Phases	Group A (2% Green Tea)		Group B (0.2% Chlorhexidine)		Group C (Plain Water)		*F value	**p-value
	#Streptococcus mutans count (CFU/ml)		#Streptococcus mutans count (CFU/ml)		#Streptococcus mutans count (CFU/ml)			
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation		
Phase 1(Before Rinse)	32.07	1.83	32.25	1.87	31.97	1.95	0.165	>0.05
Phase 2 (7 days after rinse)	24.54	1.92	24.09	1.94	31.08	1.63	135.8	<0.05

* One -way ANOVA test

**p-value<0.05 is statistically significant

[#] *Mean Streptococcus mutans count = Colony Forming Unit/ml=Total Colony Count *10^3*

Table 5a : Intergroup comparison of Mean Streptococcus mutans count (CFU/ml) among Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water) over two phases (Phase 1(Before Rinse)) , Phase 2 (7 days after rinse)

Table 5b shows pairwise comparison of mean streptococcus mutans count (CFU/ml) between groups (Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water) over two phases (Phase 1(Before Rinse)) , Phase 2 (7 days after rinse)

On pairwise comparison between Group A (2% Green Tea) and Group B(0.2% Chlorhexidine) there was no statistically significant difference in mean streptococcus mutans count(CFU/ml before and after 7 days of rinse ($p<0.05$) .

Between Group A(2% Green Tea) and Group C(Plain Water) there was no statistically significant difference in mean streptococcus mutans count(CFU/ml) before rinse($p>0.05$) but after 7 days of rinse($p<0.05$) there was statistically significant difference between them.

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Between Group B(0.2% Chlorhexidine) and Group C(Plain Water) there was no statistically significant difference in mean streptococcus mutans count(CFU/ml) before rinse($p>0.05$) but after 7 days of rinse($p<0.05$) there was no statistically significant difference between them.

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Groups	Before rinse *p-value	After rinse *p -value
Group A(2% Green Tea) : Group B(0.2% Chlorhexidine)	p>0.05	p>0.05
Group A(2% Green Tea) : Group C(Plain Water)	p>0.05	p<0.05
Group B(0.2% Chlorhexidine) : Group C(Plain Water)	p>0.05	p<0.05

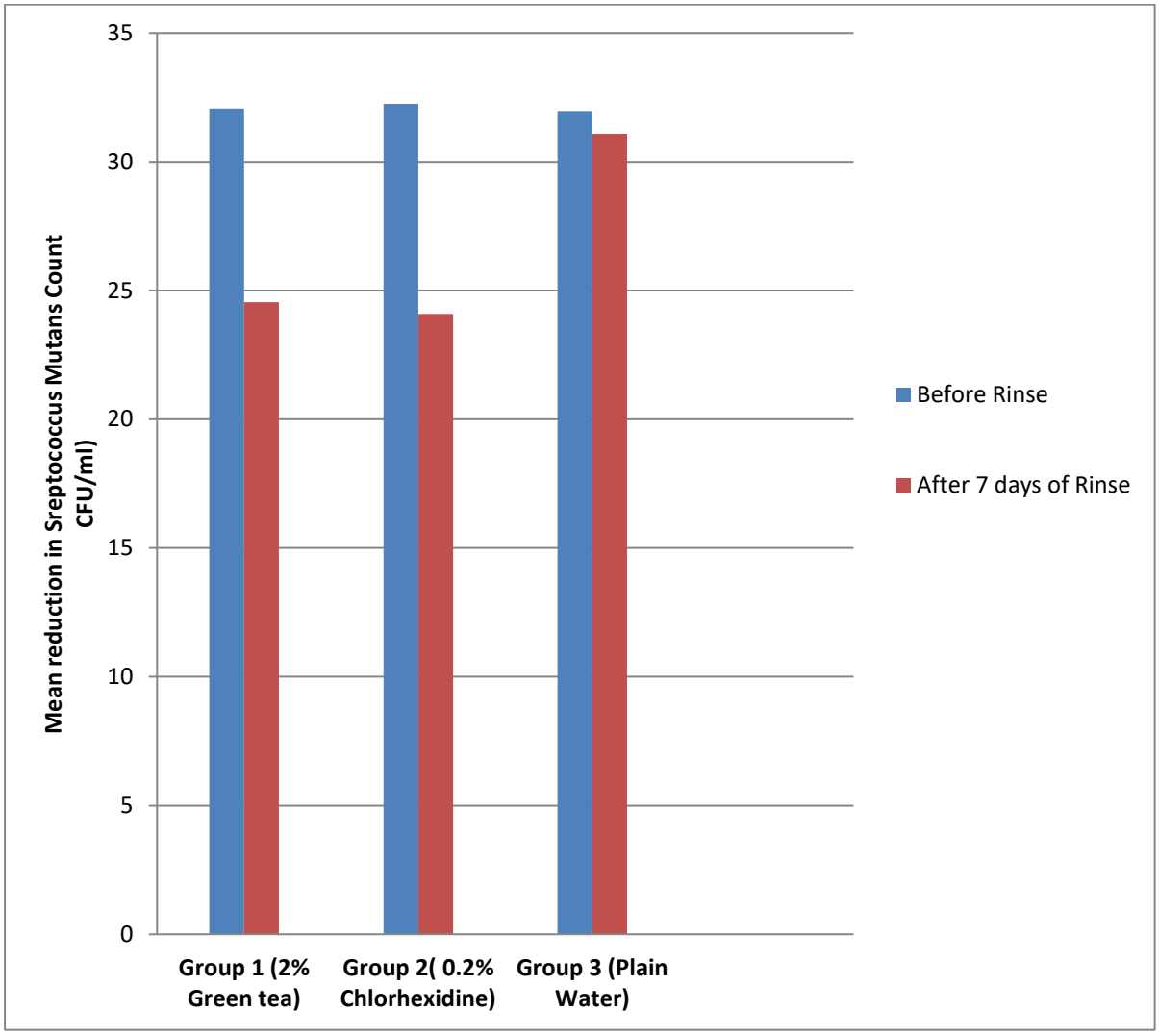
** Tukey Post Hoc test

***** p-value<0.05 is Statistically significant

Table 5b: Pairwise comparison between groups (Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water) over two phases (Phase 1(Before Rinse)) , Phase 2 (7 days after rinse)

Graph 4 shows mean reduction in the streptococcus mutans (CFU/ml) count before and after 7 days of rinse in Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) and Group C(Plain Water).

The mean reduction in the streptococcus mutans (CFU/ml) count before and after 7 days of rinse was slightly more in Group B(0.2% Chlorhexidine) in comparison to Group A(2% Green Tea) and it was least in Group C(Plain water).



Graph 4: Mean reduction in the Streptococcus mutans (CFU/ml) count before and after 7 days of rinse in Group A(2% Green Tea) , Group B(0.2% Chlorhexidine) , Group C(Plain Water)

Discussion

Discussion :

Dental caries is an ubiquitous disease affecting people since time immemorial. Dental caries is the most common chronic disease in children and the leading cause of tooth loss in adults. It causes specific bacteria to proliferate throughout the mouth. It is a multifactorial condition that is strongly associated to the presence of cariogenic bacteria, frequent use of fermentable carbohydrates and poor oral hygiene that leads to deposition of plaque and calculus. [27]

Dental plaque is made up of a diverse microbial community that grows as biofilm. It is embedded in a matrix of host and bacterial polymers . [27] Mature dental plaque with a high microbial density community containing a variety and diverse group of microorganisms. [28]

The initiation and progression of dental caries depends on oral bacteria which is involved in the fermentation of dietary carbohydrates to produce acid. Although a wide spectrum of acidogenic microbial flora may be involved . But the presence of acidogenic bacteria mainly Streptococcus mutans within plaque is responsible and is considered significant in the initiation of dental caries . Streptococcus mutans is a gram-positive , facultative anaerobic bacteria that acts on fermentable carbohydrates resulting in breakdown of fermentable carbohydrate and acid production leading to demineralization of inorganic substances and dissolution of organic solutes . The best way to prevent and reduce the development of dental caries is to reduce cariogenic bacteria such as Streptococcus mutans in the oral cavity .

Antiseptic chemical containing mouth rinse are commonly prescribed antibacterial agents as a supplement to mechanical plaque removal by dental professionals to reduce plaque which harbors wide varieties of cariogenic bacteria . The use of an effective antiseptic mouth rinse on a daily basis is generally regarded as a simple strategy that most patients can easily incorporate into their home care routine . [29]

Among different varieties of antiseptic mouth-rinses bis-biguanide chemical containing chlorhexidine is considered as gold standard mouth rinse. It is a broad spectrum cationic antimicrobial agent . chlorhexidine mouth wash is considered as gold standard due to its high substantivity and antiplaque effect in comparison to other . [30] So far, this mouth rinse is the most effective and well-documented antiplaque agent. [31] But it has various side effects associated with its long term use such as staining of teeth , mucosal lesions along with soreness and squamation of oral mucosa and alteration in taste perception . [32.33]

Hence , nowdays an increased focus is given to the use of herbal products obtained from natural sources like plants with antibacterial and anti-inflammatory activities . Green tea (Camellia sinensis) is one of the many herbal extracts with numerous medicinal benefits due to its antibacterial and antioxidant properties. [34] , [35] Green tea comprise of a larger number of bioactive chemicals that is rich in flavonoids which includes catechins . [36]

Catechins in green tea is a mixture of epigallocatechin gallate (EGCG), epicatechin gallate, epicatechin, and eppigallocatechin. [37]

Eppigallocatechin is the most abundant catechin present in green tea that is the component which is responsible for the beneficial effect conferred by green tea . [38] Various in vitro-studies have demonstrated that green tea has various antioxidant, anticarcinogenic, anti-inflammatory, thermogenic, probiotic, and antimicrobial properties . [39] Various previous studies have shown that green tea inhibits ,acid production, metabolism, growth and glucosyltransferase enzyme activity of Streptococcus mutans thereby pose deleterious effects on it by reducing its mumber . [40]

Considering this the present study was conducted to evaluate the effects of rinsing with green tea on the Streptococcus mutans count in plaque and to compare its efficacy with that of chlorhexidine mouth wash and plain water.

In the present study students of age group 18-25 years were selected for the study The participants were randomly allocated to 2% green tea mouth rinse group , 0.2 % chlorhexidine mouth wash and plain water group . The three intervention mouth rinse used in the present study was similar to the intervention mouth rinse used in the study conducted by Neturi RS et al [19] and Singh O et al [26] with difference in the concentration of chlorhexidine mouth wash which was taken as 0.12% in both studies .

In the study conducted by Singh O et al [26] Colgate Plax green tea mouth wash which is readily available in the market was used which was different from the green tea mouth wash used in the present study which was prepared manually by boiling 2g green tea bag in 100 ml of hot water for which green tea bags(Lipton green tea) that is readily available in the market was used.

In the present study participants were included in the study on the basis of plaque index given by Silness and Loe . [41] Participants with plaque score 1 were selected for the intervention .

Green tea mouthrinse and chorhexidine mouthrinse decreased plaque score when used regularly for 7 days . [5] Considering this in the present study the time period for which the participants were given intervention was 7 days. It was similar to the study conducted by Kaur H et al [5] , Ferrazzano GF et al . [15] . The study conducted by Kaur H et al [5]. further concluded that participants were kept on a follow-up period of 7 days. In a similar study conducted by Kamath N et al [41] , Nandan N et al [25] the time period for which the participants were subjected to intervention mouth rinse group that is green tea group and chlorhexidine mouth rinse group was 15 [7] days and 21 days respectively [20] and showed similar effects on cariogenic bacterias in plaque similar to the study conducted with 7 days[5] ,[42] follow-up period .

The participants were instructed to rinse for 30 seconds with the allocated mouth rinse after 30 minutes of brushing The participants were further refrained from using medicated prophylactic tooth paste and from drinking

coffee and tea 1hour after the use of mouth -rinse as it may neutralize the beneficial effect of mouth rinses which was similar to the study conducted by Kaur H et al [5].

Chlorhexidine mouthwash has been found to be beneficial in decreasing plaque and gingivitis in a study conducted by Axelsson P et al .[43] Chlorhexidine has been proven to be particularly efficient against Streptococcus mutans in plaque by Menendez et al. [44] In a study conducted by Salehi et al antibacterial effects of persica mouthwash was compared with that of standard chlorhexidine on Streptocccus mutans count . [45] Taking a queue from these studies the gold standard mouthwash was used as positive control in the present study to assess the effect of green tea mouth rinse on Streptococcus mutans count in plaque.

In the present study a significant reduction on intragroup comparison in the Streptococcus mutans count in plaque after 7 days of rinsing with green tea and chlorhexidine mouth rinse was observed . With plain water least and statistically non-significant reduction in Streptococcus mutans count was observed . The findings were similar to the study conducted by Neturi RS et al. [19] Singh O et al [26] , Nandan N et al [25] in which similar effect was observed on rinsing with green tea and chlorhexidine mouth rinse. But in these studies the time duration for which the participants were given intervention differed which was 5 minutes , 14 and 21 days .

Not many studies are mentioned in the literature on the efficacy of green tea mouth rinse on Streptococcus mutans count in plaque considering the same concentration of green tea mouthrinse used in the present study .

On intergroup comparison chlorhexidine showed greater reduction in the Streptococcus mutans count in plaque in comparison to green tea and it was least with plain water after 7 days of rinse . The reduction in Streptococcus mutans count in plaque was statistically significant on intergroup comparison after 7 days of rinse. . The findings was similar to the study conducted by Neturi RS et al. [19] Singh O et al [26] , Nandan N et al [25] , Jennifer F et al [46] , Biswas S et al [47] Mathur A et al [48] and Radafshar G et al [49] which showed the similar effect on Streptococcus mutans count in plaque on rinsing with green tea , chlorhexidine and plain water as in the present study .

On pairwise comparison between green tea mouth rinse and plain water , chlorhexidine mouth wash and plain water a statistically significant reduction in streptococcus mutans count was observed after 7 days of rinse with the mouthwashes .

Considering the findings of the present study green tea mouthrinse and chlorhexidine mouthwash are highly effective in reducing Streptococcus mutans count in plaque in comparison to plain water. The findings of the present study was similar to the study conducted by Neturi RS et al [19] Singh O et al [26] , Nandan N et al [25] which showed statistically significant reduction in Streptococcus count in plaque on pairwise comparison between green tea mouth rinse and plain water , chlorhexidine mouthwash and plain water .

On pairwise comparison between green tea mouthrinse and chlorhexidine mouth wash chlorhexidine showed more reduction in Streptococcus mutans count in comparison to green tea mouthrinse but there was no statistically significant difference between them which showed that green tea mouthrinse is equally effective in reducing Streptococcus mutans count as chlorhexidine mouth wash . The findings of the study was similar to the study conducted by Neturi RS et al. [19] Singh O et al [26] , Nandan N et al [25]

Considering the findings of the present study and herbal properties of green tea mouthrinse , it can be used as an alternative to chlorhexidine mouth wash due to its beneficial antibacterial effect on cariogenic bacteria that is Streptococcus mutans in plaque without any side effects which is commonly seen with conventional chemical containing chlorhexidine mouth wash .

Conclusion

Conclusion

The present study was conducted to evaluate the effects of rinsing with green tea and to compare its efficacy with that of chlorhexidine mouth wash and plain water on the Streptococcus mutans count in plaque.

Green tea mouth-rinse proved to be highly efficacious antibacterial herbal mouth- wash in reducing Streptococcus mutans count in plaque . With antibacterial properties it also has anti-inflammatory and antioxidant properties which also confers various positive effect on oral tissues .

In the present study the reduction in Streptococcus mutans count was more in Green Tea group and Chlorhexidine group in comparison to plain water group .Both green tea and chlorhexidine mouth wash are effective and superior antibacterial agent as compared to plain water .

After rinsing with green tea mouth rinse the reduction in Streptococcus mutans count was less but was almost equal to chlorhexidine mouth wash .

Chlorhexidine proved to be still superior mouthwash in providing antibacterial effect but it has various side effects on its long term use .

Hence with herbal properties and without any reported side- effects green tea mouth rinse can be used as an adjunct to mechanical plaque removal for longer period of time . Considering this it can be used as an alternative to conventional chemical containing chlorhexidine mouth wash on routine basis to provide antibacterial effect

Limitation

Limitations:

The study assessed the antibacterial effect of green tea mouth-rinse in plaque only on one bacteria that is Streptococcus mutans without considering its effect on other bacteria such as Lactobacillus , Aggregatribacteractinomycetes comitans , Actinomyces viscous which are some other disease causing bacteria in plaque .

The efficacy of green tea mouth rinse was evaluated for a very short follow –up period of 1 week.

The study included participants only on the basis of plaque score without considering the Decayed , Missing , Filled teeth status of the participants

Hawthrone effect due to change in oral hygiene practices of the participants out of being observed by the investigator of the study can lead to bias result.

Recommendation

Recommendations :

More extensive studies should be done involving more number of participants from different age groups with longer follow-up period .

Studies should also be done including Decayed , Missing , Filled teeth Score of the participants to evaluate the antibacterial effect of green tea mouthwash on incidence of dental caries .

Comparative Studies should be done to evaluate the efficacy of green tea mouthrinse at different concentration and its effect of green tea on different oral microbes.

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Annexure

BABU BANARASI DAS COLLEGE OF DENTAL SCIENCES
(FACULTY OF BBD UNIVERSITY), LUCKNOW

INSTITUTIONAL RESEARCH COMMITTEE APPROVAL

The project titled **"To Evaluate the Effect of Rinsing with Green Tea on Streptococcus Mutans Count in Plaque and to Compare the Efficacy of Green Tea with that of Chlorhexidine Mouth Wash and Plain Water on the Streptococcus Mutans Count in Plaque: A Randomized Control Trial."** submitted by **Dr Shraddha Mishra** Post graduate student from the **Department of Public Health Dentistry** as part of MDS Curriculum for the academic year 2019-2022 with the accompanying proforma was reviewed by the Institutional Research Committee present on **15th October, 2020** at BBDCODS.

The Committee has granted approval on the scientific content of the project. The proposal may now be reviewed by the Institutional Ethics Committee for granting ethical approval.

16/10/2020

Prof. Vandana A Pant
Co-Chairperson

Prof. B. Rajkumar
Chairperson

Babu Banarasi Das University
Babu Banarasi Das College of Dental Sciences,
BBD City, Faizabad Road, Lucknow – 226028 (INDIA)

Dr. Lakshmi Bala
Professor and Head Biochemistry and
Member-Secretary, Institutional Ethics Committee

Communication of the Decision of the VIII[th] Institutional Ethics Sub-Committee

IEC Code: 18 (Revised) **BBDCODS/10/2020**

Title of the Project: To Evaluate the Effect of Rinsing with Green Tea on Streptococcus Mutans Count in Plaque and to Compare the Efficacy of Green Tea with that of Chlorhexidine Mouth Wash and Plain Water on the Streptococcus Mutans Count in Plaque: A Randomized Control Trial.

Principal Investigator: Dr. Shraddha Mishra **Department:** Public Health Dentistry

Name and Address of the Institution: BBD College of Dental Sciences Lucknow.

Type of Submission: Revised, MDS Project Protocol

Dear Dr. Shraddha Mishra,

The Institutional Ethics Sub-Committee meeting comprising following four members was held on 22[nd] October, 2020.

1.	Dr. Lakshmi Bala Member Secretary	Prof. and Head, Department of Biochemistry & Nutrition, BBDCODS, Lucknow
2.	Dr. Amrit Tandan Member	Prof. & Head, Department of Prosthodontics and Crown & Bridge, BBDCODS, Lucknow
3.	Dr. Sumalatha M.N. Member	Reader, Department of Oral Medicine & Radiology, BBDCODS, Lucknow
4.	Dr. Akanksha Bhatt Member	Reader, Department of Conservative Dentistry & Endodontics, BBDCODS, Lucknow

The committee reviewed and discussed your submitted documents of the current MDS Project Protocol in the meeting.
The proposal was reviewed, comments were communicated to PI thereafter it was revised.

Decisions: The committee approved the above protocol from ethics point of view.

Forwarded by:

Lakshmi Bala 22/10/20

(Dr. Lakshmi Bala)
Member-Secretary
IEC

Member-Secretary
Institutional Ethic Committee
BBD College of Dental Sciences
BBD University
Faizabad Road, Lucknow-226028

(Dr. B. Rajkumar)
Principal
BBDCODS

PRINCIPAL
Babu Banarasi Das College of Dental Scien
(Babu Banarasi Das University)
BBD City, Faizabad Road, Lucknow-226

Case Recording Proforma

Name : **Code :**

Age/Gender **Address :**

Medical History :

Dental History:

Plaque Index (PI) :

18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28
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SCORE

Plagiarism Report

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