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"Comparative Evaluation of Effects of Rinsing with Green Tea on *Streptococcus Mutans* Count in Plaque with that of Chlorhexidine Mouth Wash and Plain Water: A Randomized Controlled Trial"

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Abstract:-

Introduction: 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.

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 Streptococcusmutans 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.*

I. INTRODUCTION

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 . 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 .[1]

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 . [2]

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. [3] The polyphenols target the virulence factors of the gram negative Streptococcus mutans and also inhibit the adherence of micro-organisms to the tooth surface [4] thereby decreasing the acid production in the oral cavity and increase the pH of the saliva . These activities confers anticariogenic properties to green tea .[5]

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.

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II. MATERIALS AND METHODS

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.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.Simple random sampling technique was used to select the subjects for the study .Inclusion Criteria of the study included both genders ,subjects of 18-25 years of age, subjects without known systemic illness, subjects with plaque score of 1 according to Silness and Loe Plaque Index .Pilot study was conducted in BabuBanarasi 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 mouthrinsesby 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.The sample size for the present study was determined scientifically. The data required for determining the sample size was obtained from a previous literature. [6] [7]

Sample size was calculated using G-power analysis software .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.Prior to the study , investigator [1] was trained and calibrated in the Department of Public Health Dentistry, BabuBanarasi 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%.ADA specification type III examination was followed.A study specific proformawas used to record and evaluate demographic details ,medical and dental history of each participant.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.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.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. The ethical clearance was obtained fromInstitutional 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 BabuBanarasi Das College of Dental Sciences ,BabuBanarasi Das University , Lucknow ,Uttar Pradesh.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.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.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 Streptococcusmutans in the plaque after administration of chlorhexidine.Plain water was used as placebo or negative control .The study is a single centre 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) namely 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 mouth-rinses 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.**In the 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 .**In the second phase** all the 90 subjects were instructed to rinse with the 10 ml of given mouth rinses for 30 seconds twice

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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.

III. 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.

IV. 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.

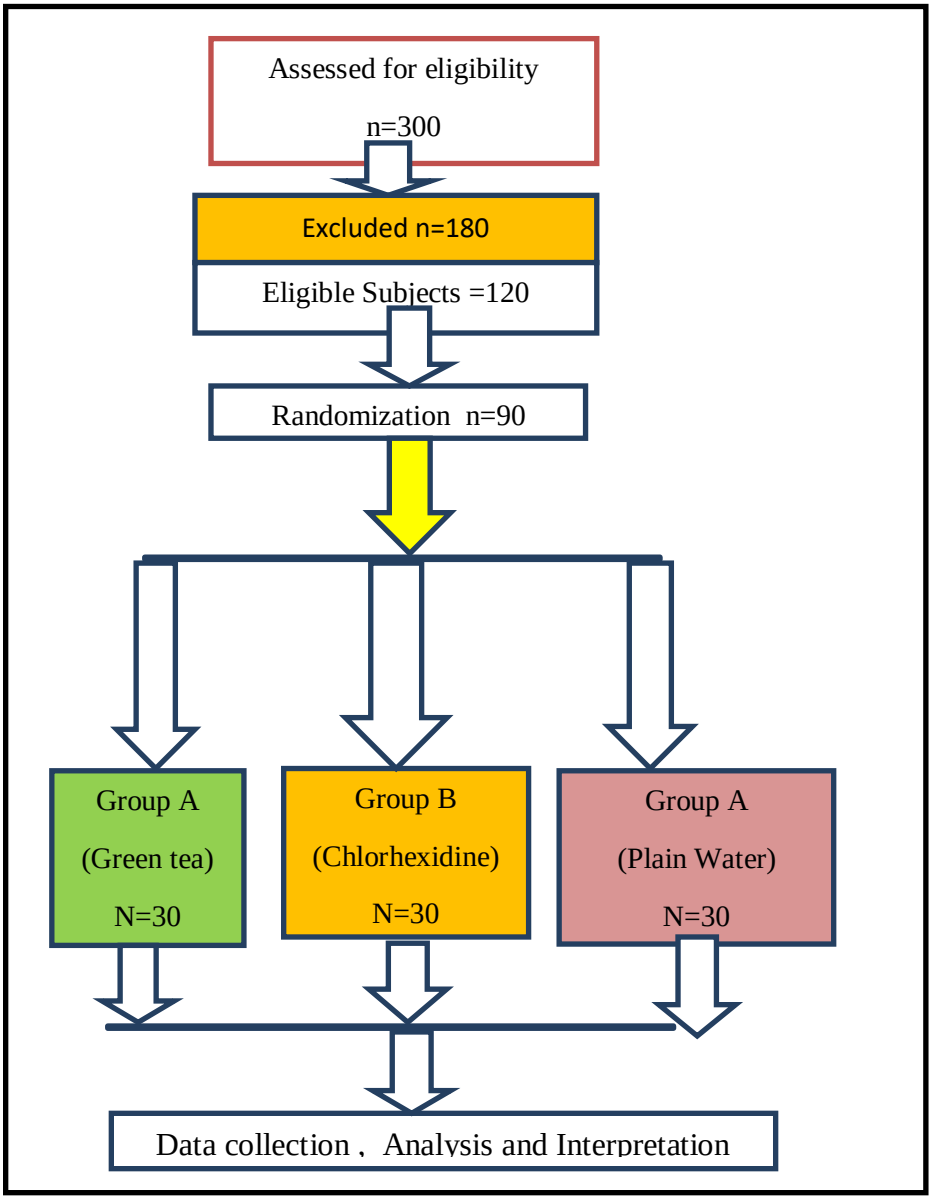

Fig. 1: Schematic representation of methodology

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V. RESULTS

Demographic details of the study participants represents that 90 study subjects participated in the study among which 30 study subjects were included in Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) , Group C (Plain(Group A(2% Green Tea) , Group B (0.2% Chlorhexidine) , Group C (Plain Water) respectively. 15 males and 15 females were included in each of the three groups.(Table 1)

On 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$\pm$1.83 and after 7 days of rinsing it was 24.54$\pm$1.92. There was a statistically significant reduction in the Mean Streptococcus mutans count before and after 7 days of rinse ($p<0.05$).In Group B(0.2%Chlorhexidine) before rinsing with 0.2% Chlorhexidine mean streptococcus mutans count (CFU/ml) was 32.25$\pm$1.87 and after 7 days of rinsing it was 24.09$\pm$ 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$\pm$1.95 and after 7 days of rinsing it was 31.08$\pm$ 1.63. There was no statistically significant reduction in the mean streptococcus count before and after 7 days of rinse with plain water ($p>0.05$).(Table 2)

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.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.(Table 3)

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					

Table 1: Demographic details of the study Participants

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

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

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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

Table 2: 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)

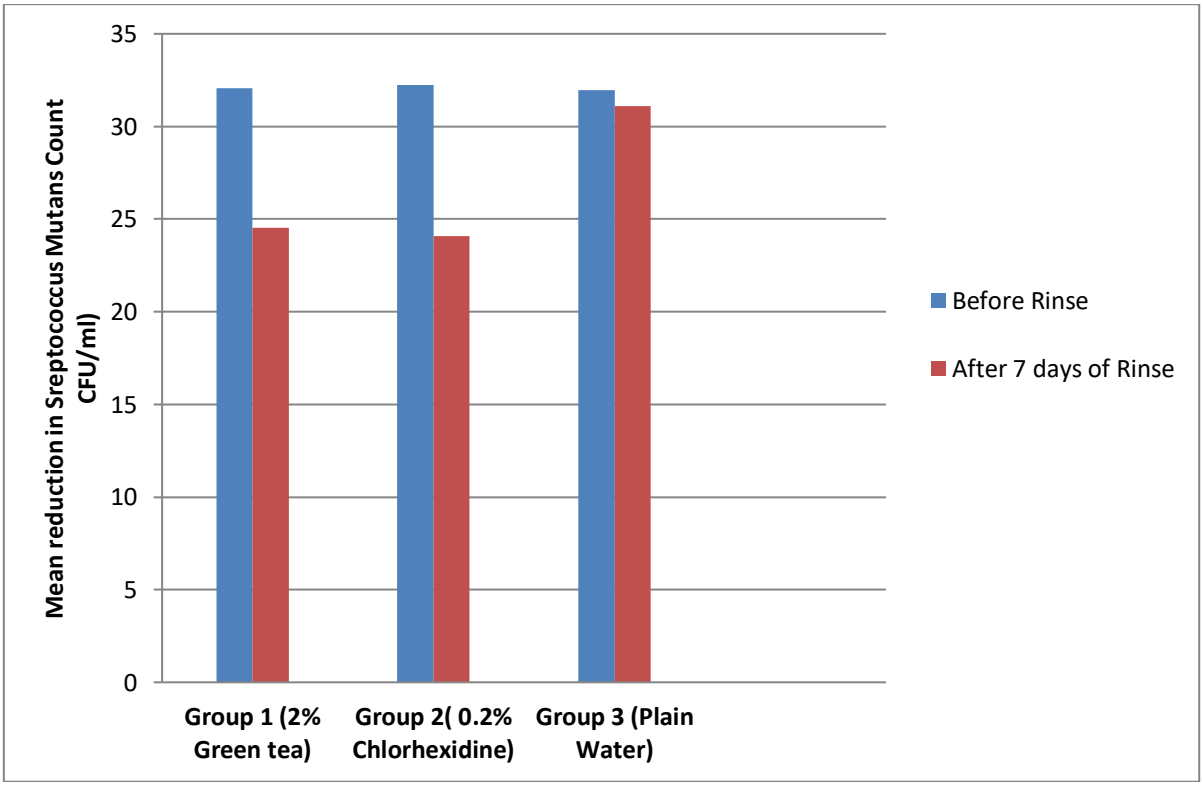
* One -way ANOVA test
**p-value<0.05 is statistically significant

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

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

Table 3: 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**)**

** Tukey Post Hoc test
*****p-value<0.05 is Statistically significant



Graph 1: 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)

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VI. DISCUSSION

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 .[8][27] Mature dental plaque with a high microbial density community containing a variety and diverse group of microorganisms. [9][28]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. [10],[11]Green tea comprise of a larger number of bioactive chemicals that is rich in flavonoids which includes catechins . [12][36]Catechins in green tea is a mixture of epigallocatechin gallate (EGCG), epicatechingallate, epicatechin, and eppigallocatechin. [13][37] Considering this thepresent 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 [6] and Singh O et al [14]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 [14] 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 . [15] Participants with plaque score 1 were selected for the intervention .Green tea mouthrinse andchorhexidinemouthrinse 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 [16] 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 [15] , Nandan N et al [17]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 [18] days and 21 days respectively [19] and showed similar effects on cariogenic bacterias in plaque similar to the study conducted with 7 days[20] .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 [20].Chlorhexidine mouthwash has been found to be beneficial in decreasing plaque and gingivitis in a study conducted by Axelsson P et al .[21]Chlorhexidine has been proven to be particularly efficient against Streptococcus mutans in plaque by Menendez et al. [22] In a study conducted by Salehi et al antibacterial effects of persica mouthwash was compared with that of standard chlorhexidine on Streptocccusmutans count . [23] 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. [6] Singh O et al [14] ,Nandan N et al [17]in whichsimilar 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 Streptococcusmutans 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. [6] Singh O et al [14] ,Nandan N et al [17], Jennifer F et al [24] , 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 [6] Singh O et al [14] ,Nandan N et al [17]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 .

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The findings of the study was similar to the study conducted by Neturi RS et al. [6] Singh O et al [14] ,Nandan N et al [17].

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.

VII. 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 washon routine basis to provide antibacterial effect .

VIII. 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 , Aggregatri bacteractinomycetescomitans , 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.

IX. 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.

REFERENCES

[1.] Rölla G, Melsen B. On the mechanism of the plaque inhibition by chlorhexidine. *Journal of Dental Research.*1975 ; 1(54): 57-62.

[2.] Shetty PR, Setty SB, Kamat SS, Aldarti AS, Shetty SN. Comparison of the antigingivitis and antiplaque efficacy of the herboral (herbal extract) mouthwash with chlorhexidine and listerine mouthwashes: a clinical study. *Pakistan Oral & Dental Journal* .2013; 33(1).

[3.] Naderi NJ, Niakan M, KharaziFard MJ, Zardi S. Antibacterial activity of Iranian green and black tea on streptococcus mutans: an in vitro study. *J Dent (Tehran)* .2011 ; 8(2) : 55-9.

[4.] Otake S, Makimura M, Kuroki T, Nishihara Y, Hirasawa M. Anticaries effects of polyphenolic compounds from Japanese green tea. *Caries Res 1991*; 25: 438–443.

[5.] Awadallaetal.A pilot study of the role of green tea use on oral health *International Journal of Dental Hygiene*, 2011 ; 9: 110–116.

[6.] Neturi RS, Srinivas R, VikramSimha B, Sandhya Sree Y, Chandra Shekar T, Kumar S. Effects of Green Tea on Streptococcus mutans Counts-A Randomised Control Trial.JCDR. 2014 Nov;8(11).

[7.] Rao A. Efficacy of Green tea Extract in Reduction of Oral Salivary Microorganisms, an in Vitro Study, *Int Journal Of Scientific Research.*

[8.] Marsh PD: Microbiologic aspects of dental plaque and dental caries. *Dent Clin North Amer*. 1999, 43: 599-614.

[9.] Socransky SS, Haffajee AD. Dental biofilms: difficult therapeutic targets. *Periodontol.*2000; 28:12-55.

[10.] Rassameemasmaung S, Phusudsawang P, Sangalungkarn V. Effect of green tea mouthwash on oral malodor. ISRN Prev Med 2013;2013:1-6.

[11.] Kondreddi S, Rao MV, Kastala VH. Crucumin and camellia: Novel indigenous armours-A pilot study to evaluate the efficacy of 5% green tea mouthwash in comparison with 0.1% turmeric mouthwash as an adjunct to oral prophylaxis in treatment of plaque induced gingivitis *Int J Recent Sci Res* 2016;7:13406-09.

[12.] Singh B and Dressings R. Gingivitis – A silent disease. *J Dent Med Sci 2013*; 6:30-3.

[13.] Kudva P, Tabasum ST, Shekhawat NK. Effect of green tea catechin, a local drug delivery system as an adjunct to scaling and root planing in chronic

periodontitis patients: A clinicomicrobiological study. *J Indian Soc Periodontol*2011;15:39-45.

[14.] Singh O, Reddy VK, Pradhan D, Sharma L. Comparative evaluation of green tea and chlorhexidine mouthwashes on gingivitis: A randomized controlled trial. J Indian Assoc Public Health Dent 2019; 17:269-74.

[15.] Kamath, N. and Kamath, S.Effect of 0.5% Green Tea Leaves (Camellia sinensis) Mouth Rinse, 0.12% Chlorhexidine Mouth Rinse and Drinking Water on Pathogens of Dental Caries in School Children in Navi Mumbai. *Int.J.Curr.Microbiol.App.Sci2018* ; 7(11): 1009-1014.

[16.] Kaur H, Jain S, Kaur A. Comparative evaluation of the antiplaque effectiveness of green tea catechin mouthwash with chlorhexidine gluconate. *J Indian Society Periodontol.*2014; 18(2) : 178

[17.] Nandan N, MythriPrasanna, Prem Kishore K. Effect of Green Tea as a Mouth Rinse on Streptococcus Mutans.*J Ayurveda Integr Med Sci 2016;*1(1):7-11.

[18.] Axelrod M, Berkowitz S, Dhir R, Gould V, Gupta A, Li E, Park J, Shah A, Shi K, Tan C, Tran MM et al . The inhibitory effects of green tea (Camellia Sinensis) on the growth and proliferation of oral bacteria .*GSJ Park*. 2010.

[19.] Rao A. Efficacy of Green tea Extract in Reduction of Oral Salivary Microorganisms, an in Vitro Study ,*Int Journal Of Scientific Research. 2014.* 3 (11): 95-6 .

[20.] Kaur H, Jain S, Kaur A. Comparative evaluation of the antiplaque effectiveness of green tea catechin mouthwash with chlorhexidine gluconate. *J Indian Society Periodontol.*2014; 18(2) : 178.

[21.] Axelsson P, Lindhe J. Efficacy of mouthrinses in inhibiting dental plaque and gingivitis in man. *J ClinPeriodontol. 1987*; 14: 205-12.

[22.] Menendez A. comparative analysis of the antibacterial effects of combined mouth rinses on streptococcus mutans. *Oral MicrobiolImmunol.* 2005; 20: 31-34

[23.] Salehi P, MomeniDanaie Sh. Comparison of antibacterial effects of persica mouthwash with chlorhexidine on streptococcus mutans in orthodontic patients. *DARU*. 2006; 14: 169-74

[24.] Jennifer F, Andriani N. Reduction of salivary Streptococcus mutans Colonies in children after rinsing with 2.5% green tea solution. *J Dent Indones*2014;21:81-6.