Research Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2020;10:2:52-60.

To Compare the Effectiveness of Two Herbal Mouthrinses (Green Tea And Guava) on Plaque, Gingival Scores among 12-15 Year Old School Children: Randomized Controlled Trial

Sweta R. Gandhi[1*] Rahul A. Gandhi[2]

[1]Assistant Professor, Department of Public Health Dentistry, VSPM Dental College, Nagpur, Maharashtra, India.
[2]Assistant Professor, Department of Oral medicine and Radiology, Saraswati Dhanwantari Dental College, Parbhani, India.

ABSTRACT

Background: Dental caries and periodontal diseases are essentially caused by the micro organisms present in dental plaque. Chlorhexidine is recognized as the primary agent for chemical plaque control. But it cannot be used on a long term basis because of various side effects like brown discoloration, taste perturbation, oral mucosal lesions, parotid swelling and sometimes unacceptable taste. An effective alternative to Chlorhexidine with all the good qualities and its unpleasant effects is highly desirable. Hence an attempt is being made in this study to compare the relative effectiveness of green tea and guava mouthwashes on plaque and gingival scores.

Materials and Methods: Two stage random sampling was done. One high school was randomly selected from the high schools of Belgaum city. All the children aged between 12-15 years were screened. A sample of 90 was randomly selected. These 90 participants were randomized into 3 groups- 30 subjects in each group. Baseline data for plaque index, gingival index was recorded for all the children selected. All the children were administered green tea, guava and Chlorhexidine mouthwashes according to the group they were assigned, twice daily for 21 days and again plaque index, gingival index were recorded.

Results: The mean baseline plaque scores and gingival scores in the Green Tea Mouthwash group was 1.512(±0.410) and 1.534 (±0.417) ,for the Guava Mouthwash group was 1.573(±0.285) and 1.590(±0.269) and for the Chlorhexidine Mouthwash group was 1.416(±0.242) and 1.446(±0.223). plaque scores and gingival scores reduced after 21 days in the Green Tea Mouthwash group 0.580(±0.291) and 0.463 (±0.181) ,for the Guava Mouthwash group was 0.753(±0.312) and 0.647(±0.268) and for the Chlorhexidine Mouthwash group was 0.620(±0.174) and 0.570(±0.126) which was statistically significant.

Conclusion: Twice daily rinsing with green tea, guava and Chlorhexidine (0.12%) mouthwashes for 21 days caused significant reductions in plaque and gingival indices. Green tea and guava mouthwashes can be recommended for use among general population and other high risk groups.

Keywords: plaque, green tea, guava, mouthwashes.

INTRODUCTION

Dental caries and periodontal diseases, the two arch criminals of oral cavity, are essentially caused by the micro organisms present in dental plaque.[1,2] Individual's susceptibility to these diseases is dependent on a complex combination of risk factors; including genetics, systemic factors, diet and oral hygiene. Control of these diseases can most readily be achieved by reducing the microbial load of the plaque bio film. [3, 4]

Mechanical hygiene procedures such as tooth brushing, interdentally brushing and dental floss

Received: Oct. 11, 2019: **Accepted**: Dec. 23, 2019
*Correspondence Dr. Sweta R. Gandhi.
Department of Public Health Dentistry, VSPM Dental College, Nagpur, Maharashtra, India.
Email: sweta.toshniwal@gmail.com

pISSN 2278-0076
eISSN 2321-9270

are the main methods for reducing plaque. However despite the potential for adequate mechanical plaque control clinical experience and population-based studies demonstrate that such methods are not being employed sufficiently by large numbers of the population. [2]

Chemical methods of reducing plaque, such as mouthwashes, are therefore appealing as they can provide significant benefits to patients who cannot maintain adequate mechanical plaque control. They can therefore be considered a less technically demanding adjuvant to mechanical control. [5] Various mouth rinses are available in the market, amongst which Chlorhexidine is the most popular. It is recognized as the primary agent for chemical plaque control, its clinical efficacy being well known to the profession.

But it cannot be used on a long term basis because of various side effects like brown discoloration, taste perturbation, oral mucosal lesions, parotid swelling, enhanced supragingival plaque formation and sometimes unacceptable taste.[3] An effective alternative to Chlorhexidine with all the good qualities and sans its unpleasant effects is highly desirable and has been long awaited.

Medicinal plants have fascinated mankind since the dawn of civilization. This is proved by the fact that all ancient systems of medicine have heavily leaned upon herbal products to treat common ailments. In the recent past, there is revival of interest in traditional medicine because it is safe, economical, and dependable and has withstood the test of time. In fact, the World

Health Organization in 2009 gave a universal call to all member states to promote safe and effective use of traditional systems of medicine. Plants have been exploited by man for many centuries as sources of chemotherapeutic and other medicinal drugs. Herbal compounds known by ancient medicine are of growing interest in the domain of oral disease prevention [6] .These herbal products are not only economical but also have less side effects are user friendly, biocompatible, safe and readily available. The natural photochemical could offer an effective alternative to antibiotics and represent a promising approach in prevention and therapeutic strategies for dental caries and other oral infections.[7] Green tea (camellia sinensis) is an evergreen tree or shrub of the Theaceae family. Mature leaves are bright green in color, leathery and smooth. Guava (psidium guajava) a native of central America is a spreading tree of the Myrtaceae family[8].Literature review revealed very few in-vivo studies worldwide assessing the effects of green tea and guava extracts on plaque, gingival scores . Hence an attempt is being made in this study to compare the relative efficacy of green tea and guava on plaque, gingival scores among 12-15 year school children of Belgaum city.

AIM

To compare the effectiveness of two herbal mouthrinses (green tea and guava) on plaque, gingival scores among 12-15 year old school children in Belgaum city.

OBJECTIVES

- To assess the effectiveness of green tea, guava and Chlorhexidine mouthrinses on plaque scores, gingival score among 12-15 years old school children in Belgaum city.
- To compare the effect of green tea, guava and Chlorhexidine, mouth rinses on plaque and gingival scores(indices) in 12-15 years old school children in Belgaum city
- To know the acceptability of green tea, guava and Chlorhexidine mouthrinses.

MATERIALS AND METHODS

Screening of school children from the randomly selected school of Belgaum city between the age group of 12-15 years was done and 90 children out of them, who meet the inclusion and exclusion criteria constituted the study sample.

INCLUSION CRITERIA

- Free from systemic diseases.
- Subjects whose gingival scores are moderate and plaque scores are fair.
- Subjects having DMFT scores between 3 and 6.
- Should not have used mouthwashes for the last 1 month.
- All the index teeth should be erupted.
- Subjects whose parents give informed consent.

EXCLUSION CRITERIA:

- Suffering from diseases which might affect the salivary flow.
- History of antibiotic therapy in the previous one month till the start of the study
- Subjects having retained deciduous teeth.
- Subjects using any mouthwashes.
- Subjects suffering from any physical or mental handicap

DROUPOUTS: Children who visit a dentist or who suffer from some systemic illness and are kept on systemic antibiotics any time during the course of study were considered as dropouts.

BLINDING: This was a double blind randomized controlled field trial. Examiner was blinded as to which mouthwash they were receiving. The statistician remained blind regarding the subject allocation to the 3 groups.

SAMPLE SIZE ESTIMATION

Based on the plaque and gingival scores, from the previous articles, sample size was calculated. The required sample size was estimated based on the difference in the plaque and gingival scores between the study and control groups. A sample size of 30 in each group was found to be sufficient.

Thus a total sample of 90 was taken.

- Following formula was used:

Based on the previous articles, sample size was calculated. [6] The required sample size was estimated based on the difference in means of *streptococcus .mutans* count between the study and control groups.

- Following formula was used:

$N = 2 S_p^2 [Z_{1-\alpha/2} + Z_{1-\beta}]^2 / \mu_d^2$
$S_p^2 = S_1^2 + S_2^2 / 2$
N= required number of sample size in each group
$S_{1=}$ standard deviation in group 1 = 0.88
$S_{2=}$ standard deviation in group 2 = 0.82
$S_p^2 = 0.88^2 + 0.82^2 / 2 = 0.7744 + 0.6724/2 = 0.7234X$
$\mu_{d=}$ mean difference between the two groups = 0.67
α= Level of significance =5%
1-β= Power of the study =80%
N= 27

27 in each group adjusted for 10% dropouts final sample estimated was 30 in each group .Thus a total sample of 90 was taken.

METHODOLOGY

2 stage random sampling was done.

- In the first stage, all the subjects were screened for inclusion and exclusion criteria.
- From among them, 90 subjects were randomly taken.
- These 90 subjects were randomly divided in three groups according to lottery method.
- Recording of baseline data was done on all the subjects, in which gingival index, plaque index were recorded.

DETAILS OF THE EXAMINATION

Clinical examination was carried out by the investigator in the school premises. The child sat on a chair comfortably while the investigator performed Type III examination using mouth mirror, explorer and CPI probe, under natural lighting conditions. A recording clerk who was trained to assist in the recording procedure helped the investigator in recording the findings.

METHOD OF RINSING

- Each child was made to rinse with 10 ml of green tea, guava or Chlorhexidine mouthrinses for 21 days twice daily for 30 seconds according to the group assigned. Rinsing was supervised by the examiner in the morning and by parents at night.
- The examiner who was performing the intervention was calibrated.
- Baseline data pertaining to the Plaque index, gingival index was obtained.
- Re evaluation of the baseline data pertaining to the Plaque index, gingival index was obtained at 21 days.

PREPARATION OF THE MOUTHWASH:

COLLECTION AND AUTHENTICATION OF MATERIAL:

- Green tea was collected from the local market in Kolhapur under the trade name “Rahimpur green tea”. Fresh Guava leaves were collected

from guava tree in Belgaum and same were authenticated by Scientist of the Regional Council of Medical Research, (ICMR), Nehru nagar Belgaum, Karnataka, India.

- After authentication guava leaves were subjected to drying at room temperature, until they were free from moisture.

PREPARATION OF GREEN TEA AND GUAVA AQUEOUS EXTRACT-

- About 500gms of dried powder of green tea and guava were subjected to cold maceration with chloroform water I.P in conical flasks separately for about 7 days at room temperature.
- The flasks were securely plugged with absorbent cotton and were shaken periodically. Then material was filtered through muslin cloth and marc was pressed.
- The filtrates were registered through whatmans filter paper to get the clear filterate.
- The filtrates were then concentrated to dry residue in a desiccators over anhydrous sodium sulphate.
- Thus pure aqueous extracts of green tea and guava were obtained.
- Based on the previous phytochemical study (in vitro and in vivo studies) the minimum inhibitory concentrations (MIC) of green tea and guava was 2 % [9] and 2 % respectively.

Colouring and flavouring agents were added to this extract to make it palatable.

EXAMINATION PARAMETERS

Plaque Index: Plaque index was described by Silness J and Loe H in 1964.

Gingival Index Gingival Index (GI) was developed by Loe H and Silness J in the year 1963, to describe the clinical severity of gingival inflammation.

RESULTS

Table 1: Shows Distribution of mean dental caries experience (DMFT) according to different mouthwash groups.

Mouthwash Group	Mean DMFT(± Std Deviation)
Green tea Mouthwash	3.59 (±0.76)
Guava Mouthwash	3.61(±0.86)
Chlorhexidine Mouthwash	4.00(±0.92)
	ANOVA p Value =0.09(NS)

Table 2 shows the distribution of the mean baseline plaque scores and gingival scores in the three groups

Mouthwash Group			Mean	Std. Deviation(±)
Green Tea Mouthwash		BL-PI BL-GI	1.512 1.534	0.410 0.417
Guava Mouthwash		BL-PI BL-GI	1.573 1.590	0.285 0.269
Chlorhexidine Mouthwash		BL-PI BL-GI	1.416 1.446	0.242 0.223

Table 3 shows the distribution of the mean plaque scores and gingival scores in the three groups After 21 days

Mouthwash Group		Mean	Std. Deviation(±)
Green Tea Mouthwash	21-PI 21-GI	0.580 0.463	0.291 0.181
Guava Mouthwash	21-PI 21-GI	0.753 0.647	0.312 0.268
Chlorhexidine Mouthwash	21-PI 21-GI	0.620 0.570	0.174 0.126

Table 4 shows the overall mean differences of the mean plaque scores and gingival scores at baseline and 21 days in the three groups.

Mouthwash Group		Mean difference	Std. Deviation(±)	t value	Sig(2-tailed)
Green Tea Mouthwash	BL-PI-21-PI BL-GI -21-GI	0.932 1.071	0.365 0.372	13.96 15.77	<.001 <.001
Guava Mouthwash	BL-PI- 21-PI BL-GI-21-GI	0.820 0.943	0.324 0.284	13.85 18.13	<.001 <.001
Chlorhexidine Mouthwash	BL-PI-21-PI BL-GI-21-GI	0.796 0.876	0.272 0.226	16.02 21.20	<.001 <.001

Table 5 shows comparison of mean plaque scores and gingival scores of three mouthwash groups at baseline and 21 days.

Time	(I) Group	(J) Group	Mean Difference (I-J)	p Value	95% Confidence Interval	
					Lower Bound	Upper Bound
Baseline Plaque Index	Green tea	guava	-0.061	1.00	-0.263	0.140
		Chlorhexidine	0.095	0.757	-0.106	0.297
	Guava	Chlorhexidine	0.156	0.185	-0.045	0.358
Baseline Gingival Index	Green tea	Guava	-0.055	1.00	-0.253	0.142
		Chlorhexidine	0.088	0.834	-0.109	0.286
	Guava	Chlorhexidine	0.144	0.239	-0.054	0.342
21 days	Green tea	Guava	-0.173	0.041	-0.341	-0.005

Plaque Index		Chlorhexidine	-0.040	1.00	-0.208	0.128
	Guava	Chlorhexidine	0.133	0.168	-0.034	0.301
21 days Gingival Index	Green tea	Guava	-0.183	0.002	-0.310	-0.057
		Chlorhexidine	-0.106	0.128	-0.233	0.020
	Guava	Chlorhexidine	0.76	0.428	-0.050	0.203

The data obtained from the study was compiled, tabulated and subjected to statistical analysis using spss version 19. The results are presented here under the headings of the various parameters considered for the study.

Number of participants At the onset of the study, there were 90 participants -30 in each group. (Green Tea=30, Guava =30, Chlorhexidine=30) on whom the final statistical analysis was done.

Distribution of the children according to gender in the study population

Green Tea mouthwash group consisted of 18 (60%) males and 12 (40%) females, Guava mouthwash group consisted of 14 (46.66%) males and 16 (53.34%) females, and Chlorhexidine mouthwash group included 17 (56.66%) males and 13 (43.34%) females.

Age wise distribution of study participants in the three groups

Mean age of participants of Green Tea Mouthwash group was 12.27 (±1.01) years, Guava Mouthwash 12.47 (±1.13)years and Chlorhexidine 12.77 (±1.16)years.

DISCUSSION

According to National Oral Health Survey caries prevalence in India was 51.9%, 53.8% and 63.1% at ages 5, 12 and 15 years, respectively. The prevalence of periodontal disease was 57%, 67.7%, 89.6% and 79.9% in the age groups 12, 15, 35-44 and 65-74 years, respectively.[10]

Both Dental caries and periodontal disease can lead to tooth loss which may interfere with normal nutrition intake, speech, self-esteem and daily routine activities. [11] Therefore Plaque control using economical and easily available chemotherapeutic agents which meet the terms with the sensibilities and pocket of the common man would be the most feasible option to promote oral health in developing countries like India.

Tea, a product made up from leaf of the plant *Camellia sinensis*, is the second most consumed beverage in the world, well ahead of coffee, beer, wine and carbonated soft drinks. Originating from China, tea has gained the world's taste in the past 2000 years. Because of the pathogenic strains (bacteria and fungi) are developing resistance to current antibiotics, there is a need for the search of new antimicrobial agents mainly among plant extracts. Psidium guajava leaves have long been recognized for their antimicrobial activity. The phytochemical analysis was observed that the Psidium guajava contains alkaloids, flavanoids, phenols and tannin which may either individual or in combination are responsible for the antibacterial activity.

The age group selected to carry out this study was 12- 15 years. Children in this age group are most vulnerable to both caries, gingival and periodontal problems due to the change in dietary habits and lifestyle.[12] Catching the children at this age has the added advantage of detecting any potential dental or periodontal disease in the early stage and avoidance of tackling an established dental disease at an older age.

Chlorhexidine has also been recognized as the positive control against which the efficacy of alternative antiplaque agents should be measure. [13] Hence Chlorhexidine mouthwash was

employed as control mouthwash in the present study.

Also Participants were selected from a high caries risk group (DMFT>3) [14] , the mean DMFT of the study sample being 3.73(±0.86) with no statistically significant difference between the three groups. Subjects with higher caries experience are known to harbour higher number of Streptococcus mutans. [15] As both caries and periodontal disease share a common etiologic agent-pathogenic microorganisms and plaque, any difference in the DMFT scores would have resulted in a bias.

Children were made to rinse with 10 ml of mouthwashes for 21 days twice daily-once in the morning in school and then again in the night at home after dinner. The time interval between these two rinsings was roughly 12 hours. This time table conformed well to the standardized regimen of Chlorhexidine mouthwash which has to be used at 12 hourly intervals as it has been shown to suppress salivary bacterial counts for over 12 hours.[13] Every participant was instructed to rinse for 30 seconds as this duration has been found optimal for intra-oral spread of mouthwashes.[16]

There were more males than females in the study sample which represented the usual gender wise distribution of children in the high schools of Belgaum. In India, males are encouraged to pursue education more than females and many females drop out of school before they reach high school.[17] This accounts for a lower female: male ratio in the study sample. Care was taken that female: male ratio was similar in each mouthwash groups.

Clinical inclusion criteria for the study were that the plaque scores should be fair and the individual should be suffering from moderate gingivitis. This group was included to provide maximum benefits of this research activity to a group which needed it and in which significant improvements could be made with a small intervention. Underlying idea was that the addition of an antimicrobial mouthrinse to daily oral hygiene regimens would help reduce the total oral bacterial burden and thereby could complement a direct action on bacteria contained within the plaque biofilm itself. [4]

Every participant was provided with 75 ml of their respective mouthwashes for home use on weekly basis. Before start of the intervention, all the children were gathered and were instructed regarding use of the mouthwashes. They were told to rinse with the mouthwashes given to them in the night before going to bed and not to eat or drink anything for atleast half an hour after rinsing. Also participants were reinforced from time to time in regular mouth rinsing.

Unstimulated saliva was preferred over stimulated saliva in this study as this unstimulated flow, is what is secreted by the salivary glands in normal physiological conditions. Unstimulated saliva is essential for the health and well being of the oral cavity and also bestows a strong protective effect to the oral cavity, against dental caries.

At baseline there was no statistically significant difference between mean plaque indices and mean gingival indices of the 3 groups (p=0.168 and p=0.061). When compared with baseline, all the three mouthwashes showed statistically significant decrease in the plaque indices and gingival indices at 21 days. After rinsing with respective mouthwashes statistically significant differences were found between green tea, guava and Chlorhexidine at 21 days,

No in vivo human study was found in literature demonstrating the effects of green tea and guava mouthwashes on plaque indices and gingival indices for 21 days. Hence comparisons could not be made. In a similar study done by Awadalla HI etal to determine the role of green tea to assess the possible protective properties of green tea on oral health , 25 subjects before and after rinsing with green tea for 5 min , Results showed that there was stastically significant difference among subjects pre and post rinsing with 2% green tea for 5 min concerning s-mutans count in saliva and plaque, salivary and plaque PH values and Gingival Bleeding Index (GBI) which supports the effectiveness of local application of green tea as antibacterial and anticariogenic material as it decreases the acidity of the saliva and plaque. [9]

Mahfuzul Hoque MD et al conducted a study to determine antibacterial activity of guava (Psidium guajava) and neem (Azadirachta indica) extracts against 21 strains of food borne pathogens. Guava

and neem extracts showed higher antimicrobial activity against Gram-positive bacteria compared to Gram-negative bacteria which suggests that guava and neem extracts possess compounds containing antibacterial properties that can potentially be useful to control food borne pathogens and spoilage organisms.[18] An in vitro study was done by S. Saraya etal, *on* Development of Guava Extract Chewable Tablets for Anticariogenic activity against *Streptococcus mutans.* The MIC against *S. mutans* of crude extract was 5mg/ml equal to minimum bactericidal concentration (MBC) which supports the traditional use of *P. guava* for the treatment of dental caries, however further study is required for this medicinal plant.[19]

Hawthorne effect cannot be overruled when discussing the results of this study.[20] As the participants of the present study were aware that they were part of an intervention, they might have improved their oral hygiene practices.

Present study was a short term study employing a crude extract of green tea and guava as mouthrinse. Though significant results were obtained at 21 days in the herbal groups, long term clinical efficacy (6 months –as prescribed by ADA)and adverse effects associated with long term usage could not be assessed. In this study, although all the children were instructed repeatedly in rinsing at home and were given daily reinforcements, some children might have failed to comply and report this failure. This might have introduced some differences in observations.

CONCLUSION

1. Twice daily rinsing with green tea, guava and Chlorhexidine (0.12%) mouthwashes for 21 days caused significant reductions in plaque and gingival indices.

2. Due to its ease of preparation and low cost, green tea and guava can be recommended for use among general population and other high risk groups.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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