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COMPARATIVE ANALYSIS OF HERBAL MOUTH RINSES IN CARIES-ACTIVE CHILDREN - A DOUBLE BLIND RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT **Introduction:** Streptococcus mutans are recognized as the primary microorganisms responsible for dental caries. Chemoprophylactic agents aim to reduce the population of S. mutans, offering an effective strategy for caries prevention. In this context, the antimicrobial efficacy of herbal mouthrinses was assessed in children. **Objectives:** The objective of this double blind randomized controlled clinical trial was to assess the effect of Green tea and Honey solutions on salivary Streptococcus mutans count in caries active children. **Methodology:** A study was conducted to evaluate the efficacy of green tea, honey, and plain water against Streptococcus mutans in a sample of 45 children aged 7 to 10 years. The participants were randomly divided into three groups, with plain water serving as the control. Unstimulated saliva samples were collected at three time points: before rinsing, immediately after rinsing, and one hour post-rinsing. The salivary S. mutans levels were analyzed using SPSS software (version 21). Statistical comparisons between the groups were performed using ANOVA and paired t-tests. **Results:** Both green tea and honey demonstrated a statistically significant reduction in Streptococcus mutans counts. A significant decrease in the mean S. mutans levels was observed from baseline to immediately after rinsing, as well as one hour post-rinse, in children assigned to the honey group ($P = 0.001$) and the green tea group ($P = 0.0001$). **Conclusion:** Green tea and Honey both are able to decrease the salivary S. mutans levels. Honey was more effective than Green tea.

KEYWORDS : Green tea, Honey, Mouth rinse, Streptococcus Mutans.

INTRODUCTION :

Carious lesion is a common illness caused by specific bacteria and dietary components interacting in plaque present on the teeth.[1] Streptococcus mutans was identified by J.K Clarke in 1924 as the primary microorganism responsible for carious lesion.[2] An important component of caries prevention programs is the use of chemoprophylactic agents that specifically target *Streptococcus mutans*. Among the various antimicrobial delivery systems available, mouthwashes have been recognized as one of the safest and most effective options—particularly in young children—due to their ability to disperse therapeutic agents across all accessible oral surfaces.[3] Antimicrobial mouthrinses such as Chlorhexidine are considered as the gold standard but due to its several side effects after long term use, interest has been generated in using herbal mouthwashes instead.[2,4] Herbal mouthwashes consist of phytochemicals that have antimicrobial and anti-inflammatory properties therefore considered highly potent without any side effects.

Green tea and Honey are potent antimicrobial agents that can have anticaries properties when used as mouthrinses.

Green tea contains multiple polyphenols and catcheins that prevent the growth of harmful bacteria in the mouth and gums. Research has indicated that green tea has several pharmacological benefits such as antibacterial, antiviral, antioxidant, anti-inflammatory, anti-cavity, and anti-aging properties.[5]

In 2018, Barroso H and colleagues reported that both green tea and black tea exhibited inhibitory effects against *Streptococcus mutans*, with complete inhibition of bacterial growth observed at concentrations of 12.5 mg/mL or higher. In contrast, the present study demonstrated that a lower concentration of green tea (10 mg/mL) resulted in a significant reduction in *S. mutans* levels when evaluated in vivo, highlighting its potential effectiveness even at reduced concentrations under physiological conditions. The study found that populations who eat more foods high in polyphenols tend to have fewer cavities.[6]

Honey has been demonstrated as an alternative to synthetic substances for prevention of tooth decay. Honey has antibacterial effect against certain bacteria, viruses and fungi. Honey's antibacterial property comes from hydrogen peroxide, flavonoids, and high sugar content.[7,8]

Therefore, the aim of this study was to compare the effectiveness of two natural mouthwashes in reducing *Streptococcus mutans* counts among caries-active children.

METHODS :

The current study was carried out in the Department of Pedodontics and Preventive Dentistry, I.D.S.T Modinagar as a double blind randomized controlled clinical trial.

The commencement of research was authorized by the Institutional Ethical Committee.The study followed the guidelines of Helisinki Declaration 2000.

The sample size for this study was calculated based on data from a previous study.[7] The minimum required sample size per group was determined to be 6 subjects. To account for potential errors and participant attrition, the sample size was increased to 15 subjects per group. Simple randomization was performed by a statistician using computer-generated random numbers to allocate participants into three groups: Green Tea (Group I), Honey (Group II), and Plain Water (Group III).(Fig.1)

Patients attending the Out Patient Department were evaluated for eligibility to take part in the research. A single examiner assessed the children and cases were chosen based on specific criteria.

Inclusion Criteria :

1. Children aged 5 to 9 years
2. DMFT/ deft $\geq$ greater than 5.
3. Children without active periodontal disease.
4. Children without ongoing orthodontic intervention.

Exclusion Criteria :

1. Children with systemic health condition or undergoing any long term medical treatment.
2. Children in need of emergency dental treatment or having active periodontal disease.
3. Physical limitation, which might preclude the mouth rinsing.
4. Children taking any antibiotics or having upper respiratory tract infection (URI) since past 1 month.
5. Any known allergy to honey or green tea.

Post-examination, the child's condition was clearly communicated to the parents to ensure understanding. They were given an explanation of the purpose of the tests and how they would be conducted. Permission was received from the parents/guardian for their participation in the research.

Preparation Of The Solution

In addition to green tea and honey, plain water was used as the control. Forty-five children were randomly assigned into three groups of fifteen each, based on the mouth rinse administered:

Group I (Green Tea): An extract was prepared by boiling 10 grams of green tea in 100 mL of distilled water for 30 minutes. The resulting solution was then concentrated to a final volume of 10 mL, yielding a 100% weight/volume (w/v) concentration.

Group II (Honey): Commercial honey (10 g) was mixed with 100 mL of boiling distilled water, boiled for 30 minutes, and reduced to 10 mL to make a 100% (w/v) solution.

Group III (Control): Plain water was used.
The honey and green tea solutions were cooled to room temperature and stored in separate sterile containers until use. The investigator remained blinded to the group allocation to minimize bias. Prior to initiating the main study, a pilot study was conducted to assess the feasibility of the prepared solutions, the study methodology, outcome measures, and statistical analysis plan. Based on the findings, practice sessions were undertaken, and necessary modifications were implemented. Intra-examiner reliability was evaluated on 10% of the sample, resulting in a kappa coefficient of 0.93, indicating excellent agreement. Participants involved in the pilot study were excluded from the main study.

Collection of Samples:
All the kids were told to eat breakfast early on the day the samples would be collected. The saliva sample was collected when the children had not consumed any food for at least 60 minutes. This measure was implemented to prevent food intake and contamination from altering the saliva composition.

The children was positioned upright on the dental chair. About 2-3ml of saliva that was not stimulated was gathered in sterile containers placed close to the mouth following a 2-minute period. (Fig. 2)

The saliva samples were collected three times :-
- Sample a -Pre rinse sample
- Sample b - post rinse sample (after rinsing with the given solution for 2 minutes)
- Sample c – 1 hour after rinsing.

The containers were labelled correctly according to the group which was allocated to them. Example : Ia, Ib, Ic, IIa , IIb , IIc etc. The samples were kept at -40^0 C untill they were delivered to the laboratory.

Technique for collecting saliva samples to measure Streptococcus Mutans levels.
Mitis-Salivarius-Bacitracin agar (Gold et al., 1973) was used as the culture medium for *Streptococcus mutans*. The inoculated plates were incubated at 37 °C for 48 hours in an environment containing 5–20% CO_2, within 4 hours of sample collection.

Colonies of S.mutans were linked by their characteristic appearance largely convex, round or globular, elevated, dark blue in color, with sizes ranging from point to pinhead, and a rough face.

Colony counts were performed using a digital colony counter, and the results were expressed as colony- forming units(CFU).(Fig. 3) All plates were processed and examined by the same researcher to maintain consistency. Confirmation of *S. mutans* was done using light microscopy.[9,10]

Statistical Analysis :
Data entry and analysis was conducted using SPSS (SPSS Inc, Chicago, Version 21.0) software. The significance level (p) value was maintained below 0.05. ANOVA and paired t-test were employed to analyze the data between the different groups.

RESULTS :
The present study aimed to compare the effects of two herbal mouth rinses on salivary *Streptococcus mutans* levels in caries-active children.

(Table 1) The baseline (pre-rinse) salivary *S. mutans* counts among the three groups showed no statistically significant difference (p = 0.154). However, analysis of salivary samples immediately after rinsing revealed a highly significant reduction in *S. mutans* CFU counts ($p < 0.0001$), with the greatest decrease observed in Group II, followed by Group I.

(Table 2) The mean *S. mutans* CFU counts immediately post-rinse were significantly different among groups (p = 0.001). One hour after rinsing, the reduction in *S. mutans* CFU remained statistically significant between groups (p = 0.0001), with Group II showing the most pronounced reduction compared to Groups I and III.

(Table 3) When comparing *S. mutans* CFU counts between pre-rinse and one hour post-rinse, the greatest reduction was noted in Group II ($3.92 \times 10^6 \pm 3.95 \times 10^6$), followed by Group I. Group III showed no significant change.

Intergroup Comparisons:
Green Tea and Honey (Groups I & II) showed statistically significant reductions in *S. mutans* immediately after rinse and sustained effect after 1 hour.Plain Water (Group III) showed no significant effect at any time point.No significant difference among groups immediately post-rinse (p = 0.154). Highly significant difference in bacterial count changes at 1 hour post-rinse (p = 0.0001), indicating honey and green tea were much more effective than water over time. **(Table 4)**

DISCUSSION
Western Uttar Pradesh is a region known for its high prevalence of fluorosis. Excessive fluoride intake has been associated with various adverse health effects, including impaired kidney function, gastro-intestinal and neurological disorders, weakened immunity, Alzheimer's disease, nausea, complications during pregnancy, respiratory issues, and damage to vital organs such as the thyroid, liver, and other endocrine glands, in addition to dental fluorosis.[11,12] As a result of these harmful effects, reverse osmosis (RO) purification systems are now commonly used to reduce fluoride levels in drinking water. However, the use of RO systems also reduces fluoride's beneficial role in preventing dental caries. Consequently, alternative strategies for caries prevention have become essential.

In this study, green tea and honey were used as mouthwashes, with plain water serving as the control rinse.

Immediately after rinsing, the levels of Streptococcus mutans in the saliva were significantly reduced by both green tea and honey. Furthermore, a continued decrease in bacterial count was observed one hour post-rinsing. Notably, honey demonstrated a highly significant reduction in *S. mutans* levels (p = 0.0001) after one hour, while green tea also showed a substantial decrease. Measuring after one hour helps assess the persistence and clinical relevance of the antimicrobial effect, which is essential for determining whether a mouthwash could be effective in real-world use.**(Table 1,2 & 3)**

Similar results were found in a 2017 study by Esian D et al. on children aged 6 to 11 to determine the association between the severity of dental caries in children and salivary levels of Lactobacillus and Streptococcus mutans. The findings highlighted a significant correlation between the level of tooth decay activity in children and the amount of Streptococcus mutans in their saliva. [13]

Recent studies have provided insights into the antimicrobial properties of green tea and honey. Green tea contains catechins, particularly epigallocatechin gallate (EGCG), which have been shown to inhibit the growth and acid production of S. mutans. Han et al. (2023) demonstrated that EGCG and other catechins significantly suppressed acid production and enhanced the efficacy of fluoride against S. mutans.[14] Similarly, Cui et al. (2023) reported that tea polyphenols and theaflavins effectively inhibited the growth of oral cariogenic bacteria, including S. mutans.[15]

Honey's antimicrobial activity is attributed to its high osmotic pressure, low pH, and the presence of hydrogen peroxide. A study by Sharma et al. (2023) found that rinsing with honey led to a significant decrease in S. mutans counts and an increase in salivary pH, suggesting its potential as a natural anticariogenic agent.[16]

A study conducted by Kalyan P et al in 2015 found that green tea with a concentration of 100mg/ml had a small zone of inhibition. This suggests that at lower concentrations, S. mutans was resistant to the effects of green tea extract. In the current study, a concentration of 10mg/ml was tested and was shown to be very effective in reducing the count of S. mutans.[17]

Honey contains bee defensin-1, an antimicrobial peptide that plays a large role in its ability to kill bacteria. Honey's antibacterial properties are primarily a result of its elevated osmotic pressure, physical characteristics, and enzymatic glucose oxidation process. The main cause of the antibacterial effect is the release of hydrogen peroxide.[18]

CONCLUSION :

Both Green tea and Honey are equally as efficient in decreasing S. mutans in children with caries and are deemed to be cost-effective in comparison to mouthwashes sold in stores. Honey achieved a larger decrease in salivary S. mutans when compared to Green tea.

TABLE

Table 1 :Comparison between Pre Rinse and immediate Post Rinse CFU of S. mutans

Groups	Pre Rinse	Immediate Post Rinse	Change in CFU from pre to immediate post rinse Mean $\pm$ SD	P- value
I	$3.43*10^{6}\pm(2.86*10^{6})$	$2.51*10^{6}\pm(2.45*10^{6})$	$0.927*10^{6}\pm(0.69*10^{6})$	0.0001
II	$4.14*10^{6}\pm(4.19*10^{6})$	$0.84*10^{6}\pm(1.12*10^{6})$	$3.29*10^{6}\pm(3.38*10^{6})$	0.002
III	$5.83*10^{6}\pm(3.04*10^{6})$	$5.80*10^{6}\pm3.04 * 10^{6}$	$0.003*10^{6}\pm(0.04*10^{6})$	0.072
P value	0.154	0.0001		

Table 2 : Comparison between Immediate Post Rinse and post one hour CFU of S. mutans

Groups	Immediate Post Rinse	Post one hour Rinse	Change in CFU from Immediate post rinse to 1 hour Mean$\pm$ (SD)	P-value
I	$2.51*10^{6}\pm(2.45*10^{6})$	$1.58*10^{6}\pm1.95*10^{6}$	$0.923*10^{6}\pm(.93*10^{6})$	0.002
II	$0.84*10^{6}\pm(1.12*10^{6})$	$0.21*10^{5}\pm0.42*10^{6}$	$.63*10^{6}\pm(0.84*10^{6})$	0.011
III	$5.80*10^{6}\pm3.04 * 10^{6}$	$5.26*10^{6}\pm3.18* 10^{6}$	$0.54*10^{6}\pm(1.87*10^{6})$	0.707
P value	0.0001	0.0001		

Table 3:Comparison between Pre Rinse and post one hour CFU of S. mutans

Groups	Pre Rinse	Post one hour Rinse	Change from pre rinse to post 1 hour rinse Mean$\pm$ (SD	P-value
I	$3.43*10^{6}\pm(2.86*10^{6})$	$1.58*10^{6}\pm1.95*10^{6}$	$1.85*10^{6}\pm(1.54*10^{6})$	0.0001
II	$4.14*10^{6}\pm(4.19 * 10^{6})$	$0.21*10^{5}\pm0.42*10^{6}$	$3.92*10^{6}\pm(3.95*10^{6})$	0.002
III	$5.83*10^{6}\pm(3.04 * 10^{6})$	$5.26*10^{6}\pm3.18*10^{6}$	$0.57*10^{6}\pm1.86*10^{6}$)	0.500
P value	0.154	0.0001		

Table 4 Intergroup Comparision of three groups

Comparison	Group I	Group II	Group III	Intergroup P value
Pre vs Immediate Post Rinse	0.0001	0.002	0.072	0.154
Immediate vs 1 Hour Post Rinse	0.002	0.011	0.707	0.0001
Pre vs 1 Hour Post Rinse	0.0001	0.002	0.500	0.0001

FIGURES AS ENCLOSED

Figure 1

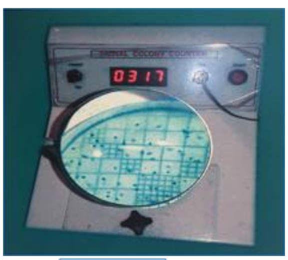

Figure 2

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