



Comparative Evaluation of Anti-Microbial Property of 5% Propolis Mouth rinse with 0.12% Chlorhexidine Mouth rinse on Streptococcus Mutans Count among 8–10-Year-Old Children

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KEYWORDS

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ABSTRACT:

Background: Dental caries is maybe the most well-known oral infection of the dental hard tissue affecting all individuals young and old. Caries susceptibility in general is much higher during childhood. The condition is a transmissible and the microorganism, Streptococcus mutans is generally implicated as the principal etiological factor. Propolis is a naturally occurring resin like substance produced by honeybees. Flavonoids, phenolics and various other aromatic compounds form its composition.

Aim: To evaluate and compare the anti-microbial effect of 5% Propolis mouth rinse and 0.12% Chlorhexidine mouthwash on S. mutans count in saliva among 8–10-year-old children.

Methodology: Sixty subjects aged 8 to 10 years were included from a school in Visnagar, Gujrat. These students were distributed into 3 equal groups (n=20) which were designated as 2 experimental groups and a control group. All students were trained to rinse with 3ml mouth rinse for 1 minute, two times a day, for 7 days under their guardian's supervision. Salivary samples were collected before using the mouthwash, after 1 hour, after 5 hour and after 7 days of use and cultured on M.S.B agar. The numbers of streptococcus mutans colonies were counted on agar medium using Digital colony counter.

Results: Both mouth rinses were highly effective in terms of reduction in S. mutans count. Inter group comparison showed both mouth rinses were equally efficacious in terms of reduction of S. mutans count.

Conclusion: Propolis Mouth wash has definite anti-microbial effect against S. mutans and can be considered as useful substitute to Chlorhexidine as an adjunct to prevent dental caries.



INTRODUCTION

The oral cavity behaves like an incubator, providing nourishment, shelter and facilitating the growth of multiple microorganisms. W.D. Miller had proposed the chemoparastic theory to explain dental caries aetiology about a hundred years ago¹. Contemporary cariogram concepts still demonstrate microorganisms as one among the foremost etiological factor for caries formation. Among them, mutans streptococci is viewed as the main culprit microorganisms.² Hence any reduction in their levels in the oral cavity will translate to reduced dental caries experience in the individual.

Multiple antimicrobials agents effective in inhibition of bacterial adhesion and colonization are available for oral use intended for inhibition of oral bacterial growth. These mouth rinses were first introduced by ancient Egyptians and Romans in addition to other oral hygiene measures. Pedanius Dioscorides (40-90 A.D), a Greek physician recommended a mixture of olive juice, pomegranate, wine, and gum myrrh to manage bad breath. Hippocrates suggested a concoction of alum, salt, and vinegar for oral rinsing.³ In recent times, Chlorhexidine mouth rinse finds favour and is generally considered the “gold standard” for its broad-spectrum antimicrobial activity. Chlorhexidine acts by destabilization of bacterial cell wall and hampers osmosis. Chlorhexidine is rapidly taken up by the bacteria, leading to cell wall rupture affecting cytoplasmic membrane and cell death.³ Nevertheless, Chlorhexidine use has been associated with unwanted effects like teeth discoloration, alterations in taste sensation, mucosal irritation and parotid swelling. It also leads to greater supra-gingival calculus secondary to precipitation of salivary proteins and organic salts which is a limitation.⁴ Several herbal products and their extracts including Guava, Neem Pomegranate, Tulsi, Green Tea, Cranberry, Grapefruit and propolis have demonstrated substantial benefits for use as mouth rinses compared chemical ones.⁵ Moreover herbal preparations are generally more readily accepted by people due to their limited or no side effects.

A substitute to Chlorhexidine, Propolis is a naturally occurring substance which has caught significant attention. The word propolis is of Greek origin from the word “pro” meaning before and “polis” meaning city to mean defender of the city.⁶ It is a resin like substance hard in consistency composed mainly of wax and plant extracts. However, the actual chemical composition of propolis varies based on geographical location it is

found, local flora specificity, plant sources, and the season it was collected.^{7,8}

Resin and balsams constitute 50-60% and pollen (5-10%). Amino acids, Vitamins A, B-complex, minerals, bioflavonoids, aromatic and phenolic compounds are other constituents.⁹ Depending on the source of origin, its colour may vary but commonly it is of brown color. Flavonoids are familiar compounds of plant origin with antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory properties.¹⁰ Propolis is dispensed as capsules, lozenges, tincture, cream. In recent times, mouth rinses, chewing gums, toothpastes and storage media for avulsed tooth are also available.¹⁰⁻¹⁴

Hence this study was designed to compare antimicrobial property of 5% Propolis with 0.12% Chlorhexidine mouth rinse on *S. mutans* count among 8–10-year-old children.

MATERIAL AND METHODS

The present study was a single-blind, randomized control trial. The study subjects included in the study were in the age group of 8–10-year-olds selected from a day school in Visnagar, Gujarat. A total 60 children were selected based on selection criteria and were randomly divided into one of the 3 groups.

Our inclusion criteria include healthy children between 8 to 10 years of age with a DMFT/dmft score of 3 to 6 following routine tooth brushing. Any child with dental abscess, a draining sinus or other dental conditions requiring emergency management or undergoing orthodontic treatment, on antibiotic therapy or suffering from any physical limitation or any issues affecting manual dexterity which might impede normal tooth brushing were excluded from the study. Children who floss or use any other oral hygiene maintenance methods apart from routine tooth brushing were also excluded.

This randomized single-blind prospective trial was conducted over a 7-day period. The trial approval was received from the ethical committee of our institution prior to the commencement of study. All the study and intervention details were shared with school authorities’ parents and participating children and parental consent obtained. An investigator was available to clarify any doubts the parents had. The diet and tooth brushing habits of children were unchanged during the duration of study.

The selected sixty children were randomized by computer generated random numbers and allocated equally into 3 groups of twenty children each as follows.



Group - A	Propolis group (5% Propolis Mouth rinse)
Group - B	Chlorhexidine group (0.12% Chlorhexidine Mouth rinse)
Group - C	Placebo group (Distilled Water)

Preparation of 5% Propolis mouth rinse

Propolis is commonly marketed as Propolis platinum (Figure - 1) [K-LINK Healthcare (India) Pvt. Ltd. Batch No. KL001-15]. It is available as 6 ml vial which contain 3.9 ml pure propolis. It was diluted in sterile water (7.7 ml propolis platinum diluted in 92.3 ml of sterile water) to a concentration of 5% mouth rinse and used for the study.

Preparation of 0.12% Chlorhexidine mouth rinse

Chlorhexidine Mouth rinse is commercially available as 0.2% concentration. [Hexidine, ICPA Health product Ltd. Batch No. L60106] (Figure – 2). 60 ml of 0.2% Chlorhexidine mouth rinse was diluted in 40 ml of distilled water and made it 0.12% and used for the study.



Figure 1- Propolis Platinum Vial



Figure 2 – Chlorhexidine Mouthwash

METHOD

The selected sixty children were divided into 3 equal groups of twenty children (n=20)

Baseline saliva sample collection:

Samples of unstimulated whole saliva was collected 1 hour after breakfast into sterile wide mouth saliva collector (2 ml on the average) before mouth rinse and collected saliva samples were stored in ice box.

Post rinse saliva sample collection:

After collecting baseline saliva samples, either Propolis mouth rinse, chlorhexidine mouth rinse or Placebo mouth rinse were given according to the group. The mouth rinse was distributed to the participants in identical container. This kept the participants blinded regarding the mouth rinse they were using. The subjects were told to rinse by either swishing 3 ml of propolis mouth rinse, 3 ml of chlorhexidine mouth rinse or 3 ml of placebo mouth rinse solution in the mouth for 60 Seconds. After 1 hour of mouth rinse and after 5 hours saliva samples were collected as mentioned earlier and sent to the laboratory to assess salivary level of S. mutans. The subjects were instructed not to eat, drink or rinse 5 hours after first mouth rinse.

Children were instructed regarding the rinsing procedure to be performed 30 minutes after tooth brushing twice daily for 7 days. The rinsing procedure was supervised by hostel warden. The subjects were instructed to avoid eating, drinking or rinsing for 30 minutes thereafter. On the 8th day morning saliva samples were collected as mentioned earlier to assess salivary levels of S Mutans.

The saliva samples were transported to the lab in ice box. Mitis Salivarius Bacitracin (MSB) agar was weighed and distilled water is added to make a uniform mixture based on the instructions given to prepare the media on the MSB agar dispensing bottle. The mixture thus obtained was autoclaved along with the culture plates. The media was poured on the autoclaved culture plates in sterilized conditions in the laboratory and allowed to set. The MSB agar plates were prepared one day prior to collection of saliva sample. The saliva samples were diluted in 0.05 M phosphate buffer (pH 7.0) to dilutions of 10^{-2} and 10^{-3} and agitated for 30 s on a vortex mixer.

Once the culture plates were prepared with the help of sterile glass spreader 50 μ l of sample was streaked on freshly prepared MSB agar culture plates for inoculation. The MSB culture plates were then incubated at 37° C for 48 hours. Colonies having



morphological characteristics of streptococcal mutans were counted in a Digital Colony Counter and expressed as number of colony forming units per millilitre (cfu/ml) of saliva.

The number of colonies per milliliter (CFU/ml) was determined by the following equation: Number of

colonies/ml (CFU/ml) = number of colonies counted \times Inverse of dilution \times inverse the cultured volume (ml).

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS 20.0 Version. Repeated measure ANNOVA test, Bonferroni Post Hoc test, Tukey HSD test and One-way ANNOVA test were used for the statistical analysis.

RESULTS

Table 1: Comparison of Mean Colony Count Among All Groups at Base Line.

Groups	Number	Mean	SD	P Value
Propolis	20	117700.00	12707.644	0.064
Chlorhexidine	20	130650.00	13739.206	
Distilled water	20	122500.00	14467.750	

Table 2: Comparison of Mean Colony Count Among Propolis Group at Various Times.

Time	Number	Mean	SD	P Value
Base line	20	117700.00	12707.644	0.000
1 hour	20	23850.00	6776.702	
5 hour	20	28400.00	5834.561	
8 days	20	34250.00	5349.619	

Table 3: Comparison of Mean Colony Count Among Chlorhexidine Group at Various Times.

Time	Number	Mean	SD	P Value
Base line	20	130650.00	13739.206	0.000
1 hour	20	20850.00	4837.083	
5 hours	20	24850.00	5203.996	
8 days	20	30750.00	6560.448	

Table 4: Pair Wise Comparison of Colony Count Between All Groups At 1 Hour.

Groups		Mean Difference	P Value
Propolis	Chlorhexidine	3000.00	0.543
	Distilled water	- 94900.00	0.000
Chlorhexidine	Distilled water	- 97900.00	0.000

Table 5: Pair Wise Comparison of Colony Count Between All Groups At 5 Hour.

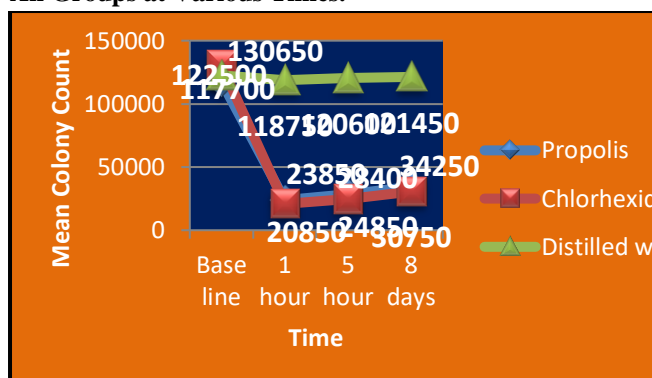
Groups		Mean Difference	P Value
Propolis	Chlorhexidine	3550.00	0.519
	Distilled water	- 92200.00	0.000
Chlorhexidine	Distilled water	- 95750.00	0.000

Table 6: Pair Wise Comparison of Mean Colony Count Between All Groups At 8 Days.

Groups		Mean Difference	P Value
Propolis	Chlorhexidine	3500.00	0.510
	Distilled water	- 87200.00	0.000
Chlorhexidine	Distilled water	- 90700.00	0.000



Graph 1: Changes In Mean Colony Count Among All Groups at Various Times.



The Mean Colony Count among all groups at base line is mentioned in Table 1. The present study results revealed a statically significant reduction in the *S. mutans* count from baseline to end of 1 hour, 5 hour and 7 days with the use of 5% Propolis mouth rinse. (Table-2). A statistically significant reduction in the *S. mutans* count from baseline to end of 1 hour, 5 hour and 7 days with the use of Chlorhexidine mouth rinse (Table-3). 5% Propolis mouth rinse and 0.12% Chlorhexidine mouth rinse significantly reduced the *S. mutans* count. In intergroup comparison at 1 hour, 5 hour and 8 days there was no significant difference between 5% Propolis and 0.12% Chlorhexidine mouth rinse on reduction of *S. mutans* count. (Table-4,5,6).

DISCUSSION

Dental caries continues to be a worldwide public health problem. The age-old concept of caries as an irreversible condition is changed. Advances in scientific technology today allow for diagnosis of the initial demineralization of tooth structure and remineralization can be achieved through a control diet, supplements of fluorides, remineralizing agents containing calcium-phosphate complexes and antimicrobial mouthwashes.¹⁵

The oral cavity harbours multiple streptococci species. One group is closely related to *S. mutans* and is commonly classified as 'mutans group' or 'mutans streptococci'. It is the most common caries causative bacteria found in the oral cavity.¹⁶ Elevated levels of salivary streptococcus mutans are generally associated with dental caries. Therefore, our study, children with DMFT/dmft between 3 to 6 were included. Caries was recorded based on the WHO Oral Health Survey 1997 criteria.¹⁷

In present study age group of 8-10 years was considered as children in this age group can rinse without inadvertent swallowing of the mouthwash and this was done to minimize any side effects. Adair proposed the prescription and use of mouth rinses in children who have mastered their swallowing reflex for the same reason.¹⁸

Propolis interferes with adhesion and glucosyltransferase activity thereby showing reductions in *Streptococcus mutans* counts. Hence it behaves like a cariostatic agent. In vivo studies confirmed reduction in *S. mutans* counts in saliva and a reduction in the plaque index.¹⁹

Our study used Propolis at a concentration of 5% as 5% Propolis showed significantly reduced *S. mutans* count.²⁰ Our findings revealed significant reduction in the *S. mutans* count from baseline to end of 1 hour, 5 hour and 7 days. This result is accordance to other study performed by Duailibe et al.²¹ and Netto C²² which had similar result.

Chlorhexidine demonstrates excellent antimicrobial activities, however multiple adverse effects like tooth discolouration, taste alterations, desquamation and oral mucosal discomfort has been reported.^{23,24} Helms et al demonstrated chlorhexidine mouthwash affected taste intensity for salty and bitter taste when used twice daily.²⁵ A concentration reduction of chlorhexidine minimizes the side effects. Hence, we used 0.12% chlorhexidine mouth wash and recorded significant reduction in the *S. mutans* count. This finding adds to the body of evidence earlier reported by Kulkarni et al² and Jayprakash R et al.²³

In this study, we revealed a statistically significant reduction in the *S. mutans* count from baseline to end of 1 hour, 5 hour and 7 days with Chlorhexidine. This result is in accordance with other studies performed by Kulkarni et al², Netto C²² and Happonen S.S. et al.²⁶. On the other hand, a study concluded that Chlorhexidine demonstrated greater anticariogenic efficacy compared to propolis against salivary *Streptococcus mutans*. However, propolis itself demonstrated a significant anticariogenic activity against *S. mutans*.²⁷ This would suggest that the effect of Propolis and Chlorhexidine mouth rinses on reduction of *S. mutans* count is similar. Children do not accept the taste of Chlorhexidine and this necessitates the exploration for an acceptable alternate agent.

The study conducted by Netto C²² showed Propolis is better than Chlorhexidine in terms of reduction of *S.*



mutans count. However, the current study result showed 5% Propolis mouth wash has similar effect as 0.12% Chlorhexidine mouth wash. This variation may be due to the different type of Propolis^{27,28} used in these studies. Propolis has been used successfully in various forms. El-Allaky et al²⁹ compared propolis delivered in 2 delivery systems namely as a mouthwash and chewing gum with both exhibiting reduced plaque scores and colony counts. Children preferred the gum over the mouthwash. Since children like chewing gum, this delivery vehicle was used for longer periods of time. They concluded Propolis in both delivery vehicles achieved reductions in plaque accumulation and microbial counts. Rodrigues et al³⁰ assessed the antimicrobial effects of a 2.5% Brazilian Red Propolis dental varnish for caries prevention in children. 2 dilutions namely 1:10 and 1:100 was evaluated and showed reduction in microbial loads, and both were found effective. Thus, propolis may be used as a mouth rinse, varnish and chewing gum providing a herbal substitute to chlorhexidine with multiple modes of administration.

Netto C²² used typified Propolis and in current study we have used Propolis Platinum. Other researchers have also found Propolis Platinum is effective against *S. mutans*.²⁰ However the current study has compared Propolis Platinum against a proven benchmark Chlorhexidine and found both are equally effective in terms of reduction of *S. mutans* count.

CONCLUSION

To our knowledge, the present study represents an initial step toward achieving an efficient, safe, and alternative natural antimicrobial mouth rinse namely Propolis. Further research in this regard could possibly deliver an effective herbal alternative to chlorhexidine available in multiple modes of delivery and with minimal to no side effects.

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