Antimicrobial Effect of Propolis Administered through Two Different Vehicles in High Caries Risk Children: A Randomized Clinical Trial

Hend S El-Allaky*/ Nadia A Wahba **/ Dalia M Talaat ***/Azza S Zakaria ****

Objective: *To investigate the effect of two methods of propolis administration on plaque accumulation and microbial count as well as patient acceptance of each vehicle.* ***Study design****: A randomized clinical trial with two parallel arms was used with a sample of 60 high caries risk children 6-8 years old. Children were divided randomly into two groups. Group I: Children who received propolis chewing gum and instructed to chew it twice daily for at least twenty minutes, for two weeks. Group II: children who received propolis mouthwash and instructed to rinse twice daily for one minute. A plaque index was recorded and a plaque sample was collected from all participants at base line and after two weeks of treatment. All participants were asked to rate the preparation they received during treatment period on a Visual Analogue Scale chart.* ***Results:*** *Data showed that propolis had a significant effect on reducing plaque scores and colony counts in both vehicles. There was no significant difference between both vehicles neither on plaque reduction nor on microbial count. However children preferred the gum formula.* ***Conclusion:*** *Propolis in both vehicles reduced plaque accumulation and microbial count which recommends its use as an antimicrobial agent in different vehicles.*

Keywords: *Propolis, Mouthwash, Chewing Gum, Antimicrobial Agent, High Caries Risk Children*

INTRODUCTION

Bacterial accumulation in dental plaque has been recognized to be the main cause of dental caries, gingival and periodontal diseases.[1] In order to maintain an ecologically favorable biofilm, oral hygiene should be improved through inhibition of plaque acid production, decrease fermentable carbohydrate consumption between meals and stimulation of salivary flow.[2]

Tooth brushing, is required for proper removal of supragingival plaque.[3] Grover *et al* (2012) [4] found that tooth brushing is an effective method to control dental plaque at home when used properly. However, effective brushing may be inefficient especially in young aged children.[5] In addition, brushing techniques show limitations in their access to interproximal plaque which necessitate the use of additional means such as dental floss.[6]

Serrano *et al* (2015) [7] and Figuero *et al* (2017) [8] reported that home care products containing chemical antimicrobials can reduce gingivitis beyond what can be achieved with brushing and flossing. Therefore, the adjunct use of antimicrobial agents offers advantages in terms of prevention of caries and gingival inflammation. [9] These products are available in different vehicles such as mouth rinse, chewing gum, oral gels, lozenges and capsules. The selection of the most adequate delivery format is of great importance.[7]

Among the various mouthwashes available, chlorhexidine is the most persistent antimicrobial agent. [10] It is considered the gold standard material for reducing plaque build up. [11] However, when used for long time, chlorhexidine mouthwash can lead to teeth staining.[12] Moreover, people with moderate to severe gum disease may not benefit from its antiseptic effect.[10]

*Hend S El-Allaky, Demonstrator of Pediatric Dentistry, Pediatric Dentistry Department, Faculty of Dentistry, Benghazi University, Libya.
**Nadia A Wahba, Professor of Pediatric Dentistry, Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Alexandria University, Egypt.
***Dalia M Talaat, Associate Professor of Pediatric Dentistry, Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Alexandria University, Egypt.
****Azza S Zakaria, Associate Professor of Microbiology and Immunology, Microbiology and Immunology Department, Faculty of Pharmacy, Alexandria University, Egypt.

Send all Correspondence to:
Dalia M Talaat
Pediatric dentistry and Dental Public Health Department.
Phone: +201221037275
Fax: +203-4871328
E-mail: daliatalaat7567@hotmail.com
Dalia.talaat@dent.alex.edu.eg

Other chemical substances can be used as antiplaque agents, most of which are associated with different side effects, accordingly; there is preference for shifting to herbal preparation which are efficient with fewer side effects.[13]

Propolis is one of the most promising natural products to prevent oral disease. It has antibacterial, antiviral, antifungal,[14] antioxidant [15] and anti-inflammatory effects [16] with little or no side effects.[17]

Propolis extract proved to be effective as an antimicrobial agent against *S. mutans*, Gram positive cocci and facultative anaerobic bacteria[18] besides its clinical value against gingival and periodontal pathogens.[19] Thus to prevent dental caries, as well as gingival and periodontal disease, it can be used as an active agent in mouthwash, however; in some cases where it is difficult to use mouthwash, other vehicles are required for oral care.[20] Chewing gum with antiplaque agent has been tested as an additional tool for daily oral care. Results showed that it can be an appropriate vehicle for the release of antiplaque agent.[21] Ercan *et al* (2015)[20] investigated the effect of propolis chewing gum compared to propolis containing mouthwash on gingival inflammation and plaque accumulation of children. Results revealed that propolis mouthwash was highly effective than using chewing gum.

Considering the importance of oral health with the potential increase in plaque accumulation and microbial colonization among children who are high caries risk, clinicians should establish the worth of self-performing antimicrobial plaque control measures as an adjunct to the mechanical measures.

Since little information is available on the best vehicle of administration that would promote children's use of propolis, this study was designed to evaluate the effect of two different methods of propolis administration on plaque accumulation and microbial count. In addition, it was important to ascertain children's preference for the method of delivery. The null hypothesis tested was that there is no difference in the clinical and laboratory outcome of two delivery vehicles.

MATERIALS AND METHOD

Study design

This study was double blind randomized clinical trial, two-group parallel arms. It was setup and reported according to the CONSORT Statement. The PICO question was '' Do high caries risk children (P) using propolis chewing gum (I) in comparison (C) to propolis mouthwash show same plaque accumulation, bacterial count, and patient acceptance for delivery vehicle after 14 days (O). Ethical approval was obtained by Dental Research Ethics Committee (#IRB NO 00010556- IORG 0008839). The protocol was registered at the National Institutes of Health (#NCT03812315). Prior to commencement of study, parent/ caregivers of all children were asked to provide an informed written consent for examination and publication, after explanation of the study's aims and procedure.

All children included in the study were with an age range of 6-8 years. They were high caries risk patients as defined by the guidelines of the American Academy of Pediatric Dentistry (AAPD), [22] free from any systemic disease and cooperative according to Frankl rating (score 3 and 4).[23] Children were excluded from the trial if they had any oral infection that compromised the mastication process or received any antibiotic two weeks before or during the study. Children with a history of using propolis containing products were also excluded.

Study setting

Children were selected from outpatient clinic of the Pediatric Dentistry and Dental Public Health Department. The material preparation and microbiological assessment were performed at the Department of Microbiology and Immunology.

Sample size estimation

The minimal sample size was calculated based on a previous study conducted by Rubido et al. (2014).[24] A sample size of 25 children per group (total sample size = 50) was the required sample to detect 0.5280 change in the primary outcome. A power of 80% was used to detect a significantly meaning difference of bacterial count reduction in dental plaque in high caries risk children receiving chewing gum containing propolis compared to those receiving mouthwash containing propolis. The estimated sample size per group was increased to 30 children per group to control attrition bias.[25]

Randomization

Subjects complying with the inclusion criteria were randomly assigned using a computer-generating list of random numbers to one of the two arms. The participants were randomly divided into two groups according to the type of treatment. Allocation was performed by a trial independent individual and the allocation ratio was intended to be equal.

Allocation concealment

Children were randomly divided into two groups. Each child included in the study was given a serial number that was used in the allocation. These numbers was written on identical sheets of paper with the group to which each child was allocated and placed inside opaque envelopes carrying the respective names of children. An independent trial personnel was assigned the role of keeping the envelopes and unfolding them only at times of giving each child the designated regimen.

Each group received a code, and the main supervisor randomly allocated the codes to the groups (I and II). Coding was done by computer software (Generate Random Codes Tools). Independent trial personnel unfolded the blinded codes at the end of statistical analysis.

Group I (N=30): children received propolis chewing gum and **Group II (N=30)**: children who used propolis mouthrinse. (Figure 1)

Blinding

The investigator and the participants were not blinded to the treatment type as each group has to be given different instructions according to their treatment protocol. However, the statistician and the microbiologist were blinded to the treatment group. .

Material preparation

Propolis chewing gum preparation

Three parts by weight of gum base was melted by heating until it softened then added to one part by weight to the previously prepared 2% propolis solution. A mixture of one hundred and five ml of 2% W/V propolis solution +2.5 g flavor + 0.3 g sorbitol + 0.3 g coloring sorbitol is added to 315 g of softened gum base.[26,27] The resultant mixture was kneaded with roll, formed and packed to the desired formula.[28] Each chewing gum piece weighted nearly 2g. This manner was repeated 4 times to reach the needed quantity.

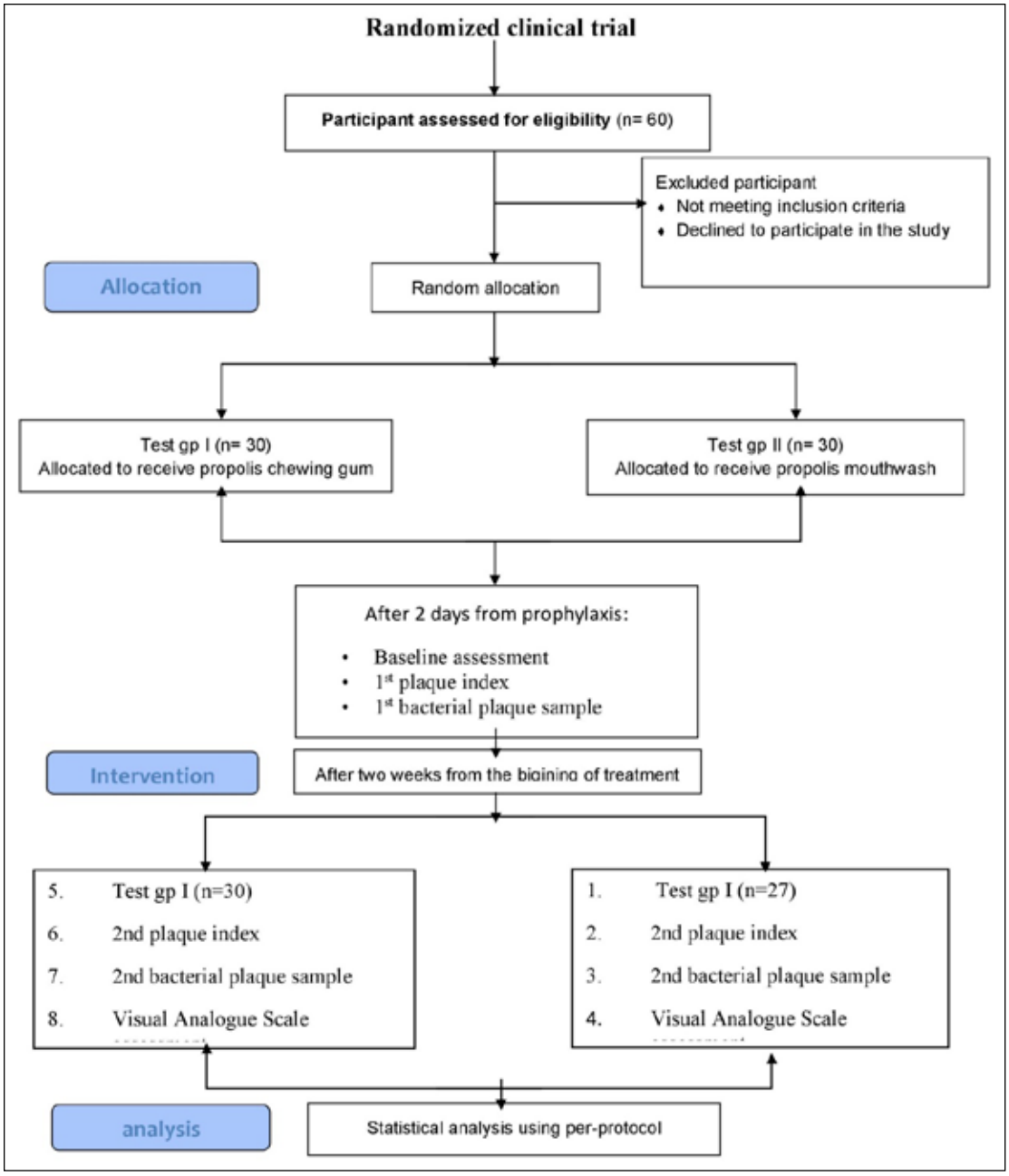

Figure 1: A CONSORT diagram showing the study protocol.

Propolis mouthwash preparation

A solution of 2% W/V of propolis was prepared by mixing 40 g propolis + 150 ml Propylene glycol + 300 ml H_2O + (60 g sorbitol + 200 ml H_2O = 40 ml flavor + 0.1 g coloring substance) + 1310 ml H_2O.[29]

Ten liters of 2% propolis solution were distributed into sterile falcon (15 ml) conical tubes. Each tube was filled to 10 ml of the prepared solution using electronic pipette pump. Each patient received 28 wrapped preloaded tubes, a tube for every single use.

Pre-test study

The propolis extract used in this study was tested by the agar disk diffusion method [30] to determine the antibacterial activity of propolis extract.

An overnight culture of standard *S. mutans* (ATCC 25175) strain was diluted to reach an approximate concentration of 0.5×10^6 CFU/mL using 0.5 McFarland standards. Then, the culture was swabbed onto blood agar plates and left to dry for 10 minutes. Next, sterile filter paper disks approximately 6 mm in diameter were immersed in 2% ethanol propolis extract. These disks were plated on the seeded blood agar plates and incubated under suitable conditions for two days. Furthermore, control disks were examined in the same manner using ethanol 70% as negative control. During this period, the test material (propolis extract) diffused into the agar and then the diameters of the inhibition zones were measured.

A moderate diameter of inhibition zones ranging from 14-17 mm were obtained, indicating a reasonable activity of the tested extract on *S. mutans* strain under examination.

Intervention

Before baseline assessment, all participants received oral prophylaxis and oral hygiene instructions including tooth brushing using the roll technique. Children were advised to brush their teeth twice daily using a soft brush and pea-sized fluoridated toothpaste.[31] On the day of sampling each child was instructed to refrain from tooth brushing in the morning, eating or drinking (except water) at least two hours before sampling procedure.[1]

Children who used chewing gum were instructed to chew a piece of gum for at least twenty minutes once after breakfast and another before bed time for two weeks. While children who used mouthwash were instructed to rinse with the whole amount present in a preloaded tube for 60 seconds twice daily once after breakfast and another before bedtime for two weeks.[32, 33] Every child was given a follow up table to be signed by his/her parent/care giver after each use of propolis chewing gum in Group I or propolis mouthwash in Group II. The table included the child data regarding the name, age, group and the serial number given to the child.

Clinical assessment of dental plaque

Assessment of dental plaque accumulation was performed quantitatively after 48 hours from oral prophylaxis and after 14 days of starting using the propolis. Plaque was estimated clinically using O'Leary, Drake & Naylor [34] Plaque Control Record. Participants were asked to swish with the disclosing solution for five seconds then spit it out. Each tooth was divided into 4 surfaces; only plaque accumulations which appeared as reddish painted surfaces were scored. The number of positively scored units was counted then divided by the total number of the evaluated teeth surfaces, and the final result was multiplied by 100 to express the index as a percentage.

Microbiological assessment of dental plaque

From each participant, a baseline plaque sample was collected after 48 hour from prophylaxis and another one was collected after fourteen days of the treatment period. Both samples were collected by running a sterile toothpick over the whole teeth surfaces. This was immediately kept in 1 ml sterile saline [35, 36] and sent for the microbiological assessment.

All samples were dispersed by vortexing for 30 seconds then 10 fold serially diluted using sterile saline. Fresh blood agar media and Mitis Salivarius agar were prepared according to the manufacturer's instructions. Then bacterial cultivation of the serially diluted samples was performed on both agar media. Finally, the plates of the media were incubated anaerobically for 48 hours before colony counting was done manually and the count was calculated as the average of two independent counts. [37]

Following the predetermined incubation period, colonies grown on the specified media were counted and represented as Colony Forming Unites (CFU/ ml) by the following equation:

Number of colonies/ml (CFU/mL) =

$$\frac{\text{Number of colonies counted} \times \text{the dilution factor}}{\text{volume taken in ml (0.02)}}$$

Assessment of participant's acceptance for the delivered treatment

On the day of the 2nd plaque sample; after completion of treatment regimen; the participants, with the aid of their parents/care givers, were asked to rate the type of treatment they received by using Visual Analogue Scale chart (VAS).[38] Each patient received the scale form and was instructed to place a vertical mark according his/ her personal rating of the preparation received during the treatment period on a horizontal line scaled from 0 to 10 where 0 represented unacceptable and 10 acceptable.

Statistical analysis

The data were analyzed by the use of SPSS software (SPSS version 25.0). Data were reviewed to check for any errors during data entry. Descriptive statistics were performed using frequencies and percentages for qualitative data (Gender and distribution of study participants regarding changes in different microbial plaque) while mean and standard deviation (SD) were used for quantitative data (Age, plaque index and $\log_{10}$ values of different microbial plaque). Normality was checked using descriptive statistics, plots (histogram and box plot) and Kolmogorov-Smirnov test. The level of statistical significance was set at 0.05.

Per protocol analyses were followed.[39] Differences between both groups were analyzed using Student's t test or Mann Whitney U test for normally and not normally distributed data, respectively. Percentage change of log values was calculated according to the formula [(final assessment-baseline assessment)/baseline assessment] x 100. Paired t test or Wilcoxon Sign Rank test were used to compare baseline and final assessment for intragroup comparisons.

RESULTS

A total of 60 children fulfilled the inclusion criteria and were recruited into the study. They were randomly assigned into two groups according to the received treatment type. Three participants dropped out at the final follow up. But they were not replaced as there were 10 cases added to the estimated sample size to control the attrition bias. The mean age values of the participants were (6.87±0.81). There was no significant difference regarding gender distribution among the two study groups ($p \leq 0.84$).

Both groups showed a significant decrease in the mean plaque indices at the final assessment ($P \leq 0.001$). Before intervention and after treatment there was no significant differences between the two study groups regarding the mean plaque index ($P \leq 0.08$, $P \leq 0.86$), respectively (Table 1).

Regarding the total microbial plaque count, there was a high significant difference between the mean values of the absolute total bacterial count before and after intervention in the two study groups ($P \leq 0.001$) (Fig.2 a, b). By comparing the two groups at baseline and after intervention no significant differences in the total microbial count was recorded ($P \leq 0.11$, $P \leq 54$) respectively. (Table 2)

Both groups showed statistically significant differences in absolute count of *S. mutans* after the intervention as compared to the baseline ($P \leq 0.001$) (Fig. 3a, b). However, among the comparison groups there were no significant differences in the total microbial count before and after treatment ($P \leq 0.61$, $P \leq 0.45$) respectively (Table 3).

Table 1:Comparison between group I and II regarding plaque index expressed as percentage of mean

Plaque index	Group I (n=30)	Group II (n=27)	P value
1st assessment: Mean±SD	42.16±12.86	47.37±16.52	0.08[a]
2nd assessment: Mean±SD	12.36±3.90	16.20±11.26	0.86[b]
P value	<0.001*[c]	<0.001*[d]	
Percentage change: Mean±SD	-68.33±11.84	-65.26±18.46	0.45[a]

a. Student's t test

b. Mann Whitney U test

c. Paired t test

d. Wilcoxon Signed Rank test

*: Significant difference p<0.05

Table 2: Comparison between group I and II regarding total bacterial count

Total bacterial count		Group I (n=30)	Group II (n=27)	P value
Baseline assessment: Mean±SD	**Absolute count**	2.26±2.50x10^7	3.67±3.64x10^7	0.11[a]
	Log_{10}	6.83±0.88	7.06±0.94	
final assessment: Mean±SD	**Absolute count**	2.36±2.38x10^5	3.38±1.44x10^5	0.54[a]
	Log_{10}	4.83±0.85	4.80±1.16	
P value		<0.001*[b]	<0.001*[b]	
Percentage change: Mean±SD		-28.95±10.66	-31.65±14.62	0.42[c]

a. Mann Whitney U test

b. Wilcoxon Signed Rank test

c. Student's t test

*: Significant difference p<0.05

Table 3: Comparison between group I and II regarding S. mutans

MS count		Group I (n=30)	Group II (n=27)	P value
Baseline assessment: Mean±SD	**Absolute count**	4.04±7.95x10^6	2.54±3.00x10^6	0.61[a]
	Log_{10}	5.39±2.01	5.87±0.95	
Final assessment: Mean±SD	**Absolute count**	0.57±1.19x10^4	0.30±1.25x10^6	0.45[a]
	Log_{10}	1.53±1.94	2.05±2.36	
P value		<0.001*[b]	<0.001*[b]	
Percentage change: Mean±SD		-64.85±38.30	-66.47±37.84	0.89[a]

a. Mann Whitney U test

b. Wilcoxon Signed Rank test

Using Mann Whitney U test, mean scores of (VAS) revealed a significant difference in the mean value of propolis chewing gum group as compared to that in propolis mouthwash group (9.50±0.86,5.78±2.17) respectively (P≤0.001).

DISCUSSION

The main objective of this study was to investigate the anti-plaque and antimicrobial effect of propolis incorporated in chewing gum and mouthwash formulation of high caries risk children. In addition children's preference of the delivery vehicle was evaluated.

Results from the present study revealed a significant reduction in plaque scores after using propolis in both vehicles in all examined participants with no significant difference between the two study groups. This reduction endorses the fact that propolis can be considered an antiplaque agent. This was supported by a study conducted by Savita *et al* [19] who showed evidence of the efficacy of mouthwash containing propolis on plaque accumulation. In addition, Ercan *et al* [20] reported significant decrease in plaque index scores in children after using propolis chewing gum. However, the reduction was less significant compared to that in children who used propolis

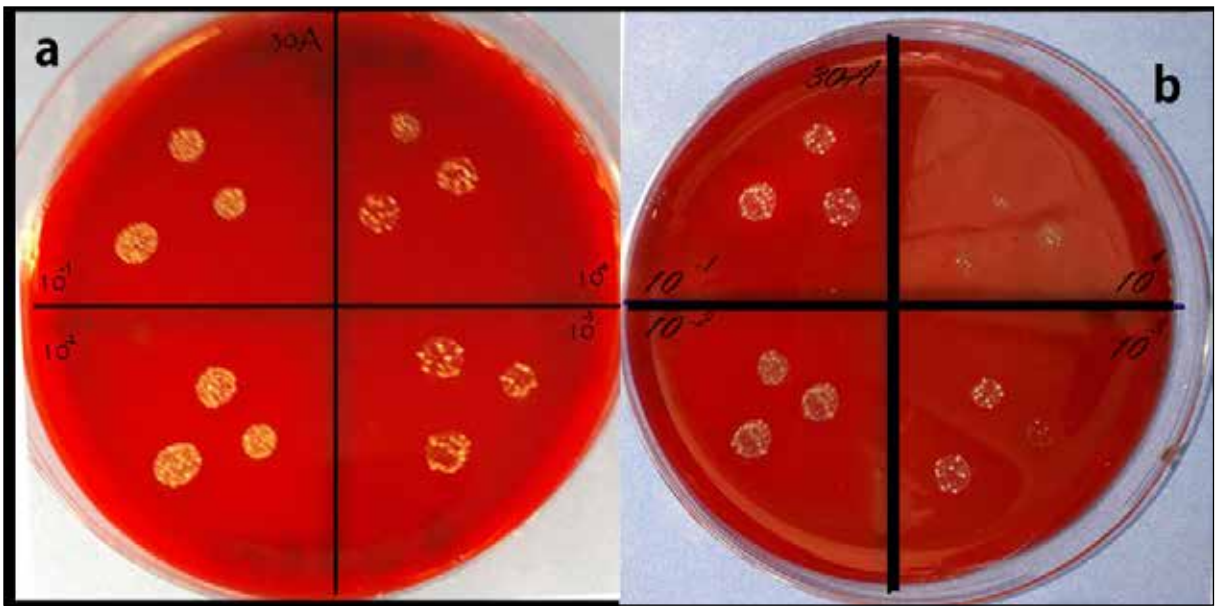

Figure 2: Microbial cultivation of plaque samples on blood agar media, (a): before intervention, (b) after intervention

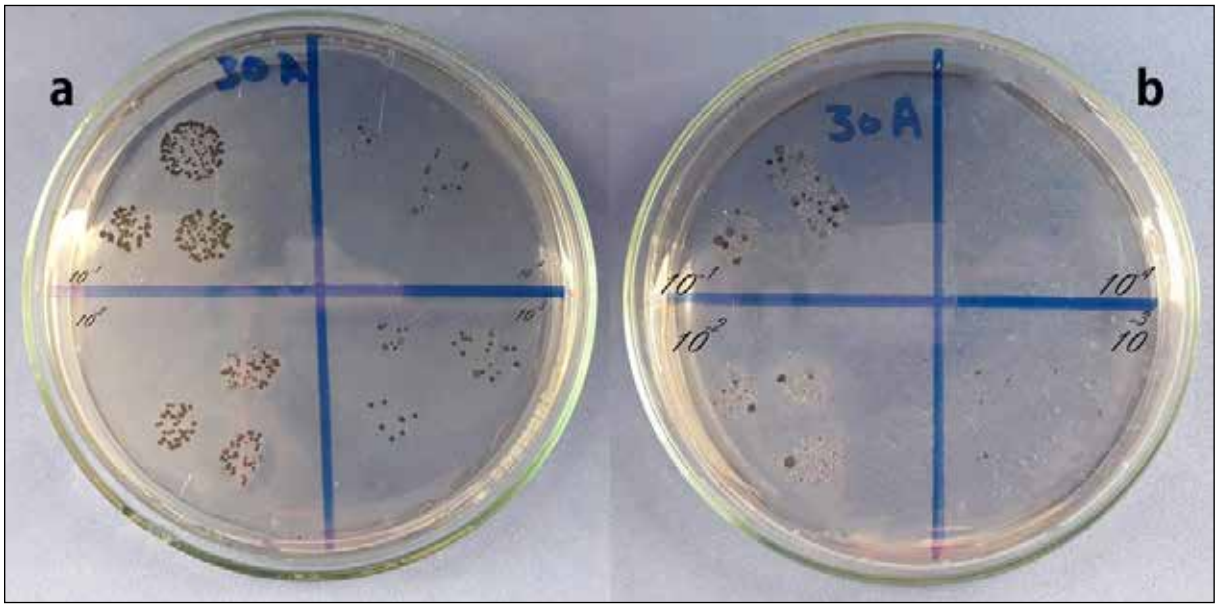

Figure 3: Streptococcus mutans colonies on mitis salivarius agar media, (a): before intervention, (b): after intervention

mouthwash. This conflict with our study could be attributed to the small sample size and short intervention period in their study. An additional variable to consider is the time of administration of each vehicle, where the time of release of propolis from chewing gum may require more than the twenty minutes set in the study.

Within group comparison, plaque samples showed significant percent reduction in absolute colony count of *S. mutans*. These results highlight the antimicrobial effect of propolis extracts. Conversely , Duailibe *et al* [40] found that half of the collected samples showed an increase or no changes in S. mutans after using mouthwash containing propolis. This variation between the studies could be due to difference in study design, study period or age of the participants.

A visual analogue Scale form was used to evaluate the acceptance of patients to the received treatment vehicle. Its simple design makes it easily to be understood and used by the targeted age group of the study. Results showed that participants were more compliant and highly preferred using propolis chewing gum more than the mouthwash, which reflects that they may use the chewing gum for longer period to achieve maximum benefits.

However, there was no significant difference among group comparison, chewing gum and mouthwash containing propolis, neither in plaque scores nor in microbial count. Results may be affected by the short trial period. Also participant compliance is considered a factor of limitation that could introduce bias and affect the trial results.

Within the limitation of this study, propolis established to have a significant effect on plaque accumulation and its microbial count either when used as chewing gum or mouthwash, despite children prefer using gum. The results suggest accepting the null hypothesis and support the use of propolis as antiplaque and antimicrobial agent in different vehicles.

CONCLUSION

Propolis proved to reduce plaque accumulation and its microbial count either when incorporated in chewing gum or mouthwash vehicle. Therefore, it is suggested to be used as antiplaque and antimicrobial agent.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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REFERENCES

1. Li J, Helmerhorst EJ, Leone CW, Troxler RF, Yaskell T, Haffajee AD, et al. Identification of early microbial colonizers in human dental biofilm. J Appl Microbiol;97(6):1311–18. 2004.
2. Seneviratne CJ, Zhang CF, Samaranayake LP. Dental Plaque Biofilm in oral Health and Disease. Chinese J Dent Res;14(2):87–94. 2011.
3. Coelho Leal S, Barreto Bezerra AC, Ayrton de Toledo O. Effectiveness of teaching methods for toothbrushing in preschool children. Braz Dent J;13(2):133–6.2002.
4. Grover D, Kaur G, Kaushal S, Malhotra R. Toothbrush ′A key to mechanical plaque control′. Indian J Oral Sci;3(2):62-8. 2012.
5. Gunsolley JC. A meta-analysis of six-month studies of antiplaque and antigingivitis agents. JADA;137(12):1649–57.2006.
6. Axelsson P, Albandar JM, Rams TE. Prevention and control of periodontal diseases in developing and industrialized nations. Periodontol 2000; 29(1):235–46. 2002.
7. Serrano J, Escribano M, Roldan S, Martin C, Herrera D. Efficacy of adjunctive anti-plaque chemical agents in managing gingivitis: A systematic review and meta-analysis. Vol. 42, J Clin Periodontol. Apr; 42 Suppl 16:S106-38.2015.
8. Figuero E, Nóbrega DF, García-Gargallo M, Tenuta LM, Herrera D, Carvalho JC, Mechanical and chemical plaque control in the simultaneous management of gingivitis and caries: a systematic review, J. Clin. Periodontol. Mars; 44: Suppl 18: S116–S134. 2017.
9. Van der Weijden FA, Van der Sluijs E, Ciancio SG,. Slot DE. Can Chemical Mouthwash Agents Achieve Plaque/Gingivitis Control?, Dent. Clin. North Am; 59(4): 799–829. 2015.
10. James P, Henry W, Parnel C, Harding M, Lamont T, Cheung A, et al. Chlorhexidine mouthrinse as an adjunctive treatment for gingival health, Cochrane Database Syst. Rev. 2017 . doi:10.1002/14651858.CD008676.pub2.
11. Van Strydonck DAC, Slot DE, Van Der Velden U, Van Der Weijden F. Effect of a chlorhexidine mouthrinse on plaque, gingival inflammation and staining in gingivitis patients: A systematic review. J Clin Periodontol ;39(11):1042–55 . 2012.
12. Kumar S, Patel S, Tadakamadla J, Tibdewal H, Duraiswamy P, Kulkarni S. Effectiveness of a mouthrinse containing active ingredients in addition to chlorhexidine and triclosan compared with chlorhexidine and triclosan rinses on plaque, gingivitis, supragingival calculus and extrinsic staining. Int J Dent Hyg;11(1):35–40. 2013.
13. Nair AA, Malaiappan S. The Comparison of the antiplaque effect of Aloe Vera , chlorhexidine and placebo mouth washes on gingivitis patients. J. Pharm. Sci&Res; 8(11): 1295–300. 2016.
14. Więckiewicz W, Miernik M, Więckiewicz M, Morawiec T. Does propolis help to maintain oral health? Evidence-based Complement Altern Med. 2013;2013.
15. Betances-Salcedo E, Revilla I, Vivar-Quintana A, González-Martín M. Flavonoid and antioxidant capacity of propolis prediction using near infrared spectroscopy. Sensors.;17(7):1647. 2017.
16. Mossalayi MD, Rambert J, Renouf E, Micouleau M, Mérillon JM. Grape polyphenols and propolis mixture inhibits inflammatory mediator release from human leukocytes and reduces clinical scores in experimental arthritis. Phytomedicine 15;21(3):290–97. 2014.
17. Miguel MG, Antunes MD. Is propolis safe as an alternative medicine? J Pharm Bioallied Sci ;3 (4):479–95. 2011.
18. Waldner-Tomic N, Vanni R, Belibasakis G, Thurnheer T, Attin T, Schmidlin P. The in vitro antimicrobial efficacy of propolis against four oral pathogens: a review. Dent J; 2(3):85–97. 2014,
19. Savita AM, Devi P, Varghese A, Prerana GK. Evaluation of Clinical Efficacy of Propolis in Patients with Gingivitis: A Randomized Clinical Crossover Study. ASDS;2(8):75-80. 2018.
20. Ercan N, Erdemir EO, Ozkan SY, Hendek MK. The comparative effect of propolis in two different vehicles; mouthwash and chewing-gum on plaque accumulation and gingival inflammation. Eur J Dent; 9(2):272–6. 2015.
21. Steinberg LM, Odusola F, Mandel ID. Remineralizing potential, antiplaque and antigingivitis effects of xylitol and sorbitol sweetened chewing gum. Clin Prev Dent; 14(5):31–4.1992.
22. American Academy of Pediatric Dentistry, Guideline on Caries-risk Assessment and Management for Infants, Children, and Adolescents, Clin. Guidel. Ref. Man. 2015-2016.; 37: 132–9. 2015.
23. Frankl SN, Shire FR FH. Should the parent remain with the child in the dental operatory? J Dent Child; 29(2):150–63.1962.
24. Rubido S, Fernández-Feijoo J, Limeres J, García-Caballero L, Abeleira MT, Diz P. In vivo antiplaque effect of three edible toothpastes. Med Oral Patol Oral Cir Bucal; 19(1): e88-e92. 2014,
25. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods; 39(2):175–91. 2007.
26. Hamada S, Iritani S, Miyake T, inventors; Hayashibara Seibutsu Kagaku Kenkyujo KK, assignee. Purified propolis-extract, and its preparation and uses. United States patent US 5,529,779. 1996.
27. Imfeld T. Chewing gum–Facts and fiction: Chewing gum- facts and fiction:a review of gum-chewing and oral health. Crit Rev Oral Biol Med; 10(3):405–19.1999.
28. Lakshmi SV, Yadav HK, Mahesh Kp, Uniyal S, Ayaz A, NagavarmaBVN. Formulation and evaluation of medicated chewing gum as antiplaque and antibacterial agent. J Young Pharm; 6(4):3–10. 2014.
29. Al-Hasani M, Hanno A, Dowidar K, Mostafa O, Soliman S. Effectiveness of Egyptian Propolis on Dental Plaque Formation in High Caries. Alexandria Dent J; 41(2):194–8. 2016.
30. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal;6(2):71–9. 2016.
31. Denbesten P, Hee M, Ko S. Fluoride levels in whole saliva of preschool children after brushing with 0.25 g (pea-sized) as compared 1.0 g (full-brush) of a fluoriile dentifrice. Pediatr Dent ;18(4):277–80. 1996.
32. Imfeld T. Chlorhexidine-containing chewing gum. Clinical documentation. Schweiz Monatsschr Zahnmed; 116(5):476–83. 2006.
33. Syed M, Chopra R, Shrivastava V, Sachdev V. Comparative evaluation of 0.2% chlorhexidine mouthwash, xylitol chewing gum, and combination of 0.2% chlorhexidine mouthwash and xylitol chewing gum on salivary streptococcus mutans and biofilm Levels in 8- to 12-year-old children. Int J Clin Pediatr Dent; 9 (4):313–9. 2016.
34. O'Leary TJ, Drake RB NaylorJE. the plaque control record. J Periodontol ;43(1):38. 1972.
35. Yang Qiong X, Zhang Q, Lu Ying L, Yang R, Liu Y, Zou J. Genotypic distribution of candida albicans in dental biofilm of chinese children associated with severe early childhood caries. Arch Oral Biol; 57(8):1048–53. 2012.
36. Elnakady YA, Rushdi AI, Franke R, Abutaha N, Ebaid H, Baabbad M, et al. Characteristics, chemical compositions and biological activities of propolis from Al-Bahah, Saudi Arabia. Sci Rep; 7: 41453 . 2017.
37. de Carvalho FG, Silva DS, Hebling J, Spolidorio LC, Spolidorio DMP. Presence of mutans streptococci and candida spp. in dental plaque/dentine of carious teeth and early childhood caries. Arch Oral Biol; 51(11):1024–8. 2006.
38. Cline ME, Herman J, Shaw ER, Morton RD. Standardization of the visual analogue scale. Nurs Res; 41(6):378–80. 1992.
39. Sedgwick P. Intention to treat analysis versus per protocol analysis of trial data., BMJ. 2015;6; 350: h681.
40. Duailibe SA, Gonçalves AG, Ahid FJ. Effect of a propolis extract on streptococcus mutans counts in vivo, J. Appl. Oral Sci; 15(5): 420–3. 2007.