EFFECTIVENESS OF EGYPTIAN PROPOLIS ON DENTAL PLAQUE FORMATION IN HIGH CARIES RISK CHILDREN

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ABSTRACT

INTRODUCTION: The greatest preventive challenge in dentistry is the control of dental biofilm and consequently avoiding dental caries and gingival diseases. As an adjunct to the mechanical oral hygiene measures, antibacterial agents seem to offer great benefits in the control of plaque formation and gingivitis, especially in high risk patients with orthodontic appliances.
OBJECTIVES: The aim of this study was to investigate the effect of propolis mouthwash in children with fixed space maintainers regarding quantity of dental plaque and its microbial population.
MATERIALS AND METHODS: Forty children with space maintainers with an age range of 6 to 8 years were randomly assigned into 2 groups (test and control). The test group received the propolis mouthwash, and the controls received a placebo. All the patients were examined clinically to assess plaque accumulation using the plaque control record before and after the treatment. Plaque sampling and microbiological evaluation was used to estimate the numbers of colony forming units.
RESULTS: Data showed that the controls experienced no significant reduction in microbial plaque count from baseline to the end of 3 weeks ($P \leq 0.11$) whereas in the propolis mouthwash users there was a significant difference after 3 weeks ($P \leq 0.0001$). Among groups comparison of total microbial plaque counts before intervention, showed a significant difference ($P \leq 0.02$), and after the intervention, there was no significant difference between groups ($P \leq 0.72$). No significant difference was found in plaque index scores in the controls from baseline to the end of 3 weeks ($P \leq 0.15$), whereas in the propolis group there was a highly significant difference ($P \leq 0.0001$). Comparisons of mean plaque values between test and control groups before intervention showed significant difference ($P \leq 0.04$). After intervention the difference increased dramatically to a highly significant value ($P \leq 0.008$).
CONCLUSIONS: A mouthwash containing propolis significantly reduced bacterial count and plaque accumulation when used for 3 weeks.
KEYWORDS: Propolis, plaque, children, high risk, antibacterial.

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INTRODUCTION

The etiology of dental caries and various forms of periodontal disease has long been recognized to be related to the bacterial accumulations in the dental plaque (1, 2). Rozkiewicz et al. in 2006, reported that many of the early microbial colonizers of dental plaque are of great importance in the succession stages of biofilm formation and its overall pathological effect on the oral health of the host (3). Plaque accumulation also is strongly associated with caries development in young children (4, 5).

The causes and risks of gingival diseases are as varied in children as in adults and range from local to systemic causes. The most important local predisposing factor in children is poor oral hygiene, which stems from children's dependence on adults for assistance with routine oral hygiene. It also stems from age limitation in perception of the need for regular and efficient tooth brushing (6).

It has been reported that fixed and removable orthodontic appliances, brackets and bands frequently cause gingival infection thereby complicate oral hygiene and cause inflammation, bleeding, gingival enlargement and increase in pocket depth (7).

Reducing the microbiological burden is the focus of interventions using antimicrobial rinses and dentifrices and behavioral intervention to improve oral hygiene and thus remove the bacterial plaque coating tooth surfaces (8).

Dental hygiene care incorporates antimicrobial agents as adjuncts to traditional mechanical dental hygiene procedures or nonsurgical periodontal therapy, and as a measure to reduce the risk of hematogenous infection subsequent to oral tissue manipulation (9).

Understanding antimicrobial agents is crucial to modern dental hygiene practice. The properties of these agents influence the effectiveness of medications prescribed by dentists or administered to patients (10). The main goal of antimicrobial therapy is to achieve a shift from an ecologically unfavorable to an ecologically favorable biofilm (11).

Propolis was used at the time of Egyptian and Greek civilizations, which recognized its healing qualities. The chemical composition of propolis is complex; flavonoids and (hydroxyl) cinnamic acid derivatives have been considered the primary biologically active compounds (12).

Previous in vitro studies have shown that propolis inhibited the growth of streptococcus mutans and was used in the management of dental caries, endodontic treatment and vital pulp therapy. The agent was also used in the treatment of oral lesions, periodontal infection and repair of surgical wounds (12, 13). Furthermore, propolis in drinking water or applied topically reduced the incidence of dental caries in rats (14).

Recently the global need for alternative treatment medication opened new channels to explore safer, effective and economically efficient products.

The present study was planned to investigate the effect

of propolis mouthwash in children with fixed space maintainers regarding quantity of dental plaque measured by plaque control record and plaque microbial population by estimating the numbers of colony forming units.

MATERIAL AND METHODS

This study was designed as a triple blinded randomized clinical trial to evaluate the effect of propolis mouthwash. Forty children with an age range of 6-8 years were selected from the Pediatric Dentistry department, Faculty of Dentistry, Alexandria Univeristy.

A power of 80% was used to detect a significantly meaning difference of bacterial count reduction in dental plaque in high caries risk children. The minimal required sample size was calculated to be 40 patients, 20 children in each group. The sample size was calculated using Med-Calc software (15).

The selected children fulfilled the following inclusion criteria; having fixed space maintainers and fully restored teeth, healthy free from any systemic disease or syndrome and cooperative according to Frankl rating scale (16). Children were excluded from the study if they previously used any propolis containing products, received any antibiotics 2 weeks before or during the study and developed any oral infections that compromise mastication.

The selected children were allocated randomly using the Fish Bowl technique to 2 groups, a control group (group I), which included 20 patients who used the placebo mouthwash and a test group (group II), which included 20 patients who used the propolis mouthwash.

The patient, investigator, bacteriologist as well as the statistician were blinded to the active ingredient the group was using. Each of the mouthwash and placebo received a code, and the mouthwash was randomly allocated to the groups (I and II). Coding was done by computer software (Generate Random Codes Tool). The supervisor at the end of statistical analysis unfolded the blinded codes.

This study was performed after receiving the approval of Research Ethics Committee, Faculty of Dentistry, Alexandria University and obtaining official consent from parents. All children diagnosed with any dental problem received dental treatment before beginning of the study in outpatient clinic of the Pediatric Dentistry department, Faculty of Dentistry, Alexandria University.

Intervention Procedure:

Preparation of 2% W/V solution of propolis mouthwash was manufactured in the Analytical and Pharmaceutical Chemistry Department Laboratories at the faculty of Pharmacy and Drug Manufacturing, Pharos University. Its composition consisted of the following ingredients by weight:

40 g propolis + 150 ml Propylene glycol + 300 ml H^2O + (60 g sorbitol + 200 ml H2O + 40 ml strawberry flavour + 0.1 g coloring substance) + 1310 ml H^2O.

Ten liters of 2% propolis solution were distributed into 20, 500 ml plastic bottles and labeled with instructions to patients. The instructions involved direction of use. The placebo mouthwash solution was prepared in the same way used for the propolis mouthwash without adding the active ingredient.

Before baseline assessment, all children received oral prophylaxis and oral hygiene instructions including tooth brushing using the roll technique (17).

All children were instructed to brush their teeth twice daily using a soft brush and pea-sized fluoridated toothpaste provided by the investigator.

After baseline assessment i.e. dental plaque measurement and bacterial sampling, each child was given the allocated mouthwash with its measuring cup. The children were instructed to rinse twice daily for 1 min with 10 ml of the mouthwash after breakfast and at bed time for 3 weeks. Each bottle contained 500 ml of the mouthwash to be used throughout the study period. All children were re-called every week for follow up and reinforced oral hygiene instructions.

Assessment methods of supragingival plaque:

- Quantitative assessment of supragingival plaque accumulation was carried out after 48 hours from oral prophylaxis and after 21 days. Plaque was estimated clinically using the Plaque Control Record (O'Leary, Drake & Naylor). The child was asked to chew the disclosing tablet and let it mix with saliva for 30 second and spit it out. Each tooth was divided into 4 surfaces, and plaque accumulation on all teeth was scored. The number of positively scored units was divided by the total number of tooth surfaces evaluated and the result was multiplied by 100 to express the index as a percentage (18).
- Microbiological assessment of supragingival plaque was carried out at baseline after 48 hours following prophylaxis. 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 (19). Using sterilized toothpicks, supragingival plaque sample was collected from the site of orthodontic band by running it along the gingival margin of banded molars (20, 21). Each plaque sample was inoculated in a separated vial, containing 1 ml of sterile brain heart infusion broth and the sample was sent immediately to the lab for microbial assessment. After 21 days another plaque sample was collected using the same method of baseline bacterial sampling.

Bacteriological Procedure

Microbial assessment was done on several steps. It began with the preparation of fresh blood agar media according to the manufacturer's instructions (22). Bacterial cultivation of serially diluted samples was performed on blood agar media. Before dilution, each plaque sample was shaken well by means of Vortex test tube mixer for 30 seconds to obtain a homogenous solution. A set of tubes containing 1ml of sterile brain heart infusion was prepared.

Preparation of a dilution of 1:10: Using an automatic micropipette and sterile tip, a hundred micron of each sample was obtained from the original tube which contained 1ml of the plaque sample. The solution was placed into one of the above mentioned set of tubes. The test tube was vigorously shaken well by means of Vortex test tube mixer for 30 seconds to obtain a homogenous solution.

Preparation of the final 1:1000 dilution: A hundred micron from the tubes containing 1:10 dilution was obtained and placed into a tube to get a dilution of 1:100. Test tube was vigorously shaken well by means of Vortex test tube mixer for 30 seconds to obtain a homogenous solution. An amount of one hundred micron was obtained from the last tube and placed into the final tube to get a 1:1000 dilution.

Cultivation of the collected plaque sample was done by using an automatic micropipette, 0.1 ml of the last dilution (1:1000) was taken from the test tube and placed on the center of labeled blood plates. The inoculated plates were incubated anaerobically in gas pack jar and the incubator

adjusted to 37° C for 48 hours before colony counting. The number of colonies or colony forming units (CFU) was counted by the following equation: Number of colonies/ml (CFU/ml) = Number of colonies counted ×the dilution

STATISTICAL ANALYSIS

Descriptive statistics were calculated and displayed as frequency and percentage (for gender) or mean and standard deviation (for age, absolute and log value of total plaque microbial count and plaque index). Normality of variables was assessed using Kolmogrov Smirnov test and for the non-normally distributed absolute count of total microbial plaque, log10 was calculated to normalize it. t test was used to compare the 2 study groups regarding log value of total microbial plaque and plaque index and their percent reductions. Paired t test was used to compare baseline and final values in the same group. Bar charts were used for graphical presentation. SPSS version 17.0 was used for data analysis. Significance level was set at 5%.

RESULTS

A total of 6 participants dropped out at the last follow up examination and were replaced. Following randomization, there was no statistically significant difference between the two groups regarding age and gender, (P= 0.75) and (P= 0.33) respectively.

In the control group, the mean and standard deviation of plaque index at baseline and after 3 weeks were 56.96 (10.48) and 54.50 (14.29) respectively. There was no significant difference (P≤0.15). In the test group, the mean and standard deviation of plaque index at baseline and after 3 weeks were 50.10 (10.18) and 39.05 (12.19) respectively. There was a significant difference (P≤0.0001). The mean plaque index before intervention showed a significant difference between groups (P≤0.04). After the intervention, the difference between the groups was highly statistically significant (P≤0.008). (Table 1)

In the control group, the mean percent reduction was 4.99 (13.38), whereas in the test group the mean percent reduction was 21.79 (18.90). There was a significant difference between the two groups (P≤0.002). (Table 2)

Table 1: Mean plaque index in the two study groups before and after intervention.

	Control Group (Placebo) Mean (SD)	Test Group (Propolis) Mean (SD)	P value
Before the intervention	56.96(10.48)	50.10(10.18)	0.04*
After the intervention	54.50(14.29)	39.05(12.19)	0.008*
P value	0.15	<0.0001*	

* Statistically significant at P ≤ 0.05

Table 2: Percent reduction in plaque index in the two study groups following the intervention.

	Control Group (Placebo)	Test Group (Propolis)
Mean (SD)	4.99(13.38)	21.79(18.90)
P value	0.002*	

* Statistically significant at P ≤ 0.05

In the control group, the mean and standard deviation of the total microbial plaque count at baseline and after 3 weeks was 1.82 (1.86) $\times 10^4$ and 1.17 (1.58) $\times 10^4$ respectively. The mean and standard deviation of log value at baseline and after 3 weeks were 4.07 (0.42) and 3.80 (0.51) respectively. There was no significant difference between means of the before and after intervention values (P≤0.11) In the test group, the mean and standard deviation of the total microbial plaque count at baseline and after 3 weeks were 3.26 (2.49) $\times 10^4$ and 1.10 (0.99) $\times 10^4$ respectively. The mean log value at baseline and after 3 weeks were 4.37 (0.38) and 3.85 (0.44) respectively. There was a significant difference between means of the before and after intervention values (P≤0.0001). Among groups comparisons of the total microbial plaque counts before intervention, showed a significant difference (P≤0.02) between the 2 groups. After the intervention, there was no significant difference between groups (P≤0.72). (Table 3)

The mean percentage change in microbial plaque counts in the study groups were reduced following the intervention. In the control group the mean and standard deviation percent reduction were 5.47 (17.14), whereas in the test group the mean and standard deviation percent reduction were 11.48 (11.23). There was no significant difference between groups (P≤0.20). (Table 4)

Table 3: Total microbial plaque count and log values in study groups at baseline and after three weeks (after intervention).

		Control Group (Placebo) Mean (SD)	Test Group (Propolis) Mean (SD)	P value
Baseline (Before intervention)	**Absolute count (CFU)**	1.82(1.86)× 10^4	3.26(2.49) $\times 10^4$	0.02*
	Log value	4.07(0.42)	4.37(0.38)	
After 3 weeks (After intervention)	**Absolute count (CFU)**	1.17(1.58) $\times 10^4$	1.10(0.99) $\times 10^4$	0.72
	Log value	3.80(0.51)	3.85(0.44)	
P value		0.11	<0.0001*	

* Statistically significant at P ≤ 0.05

Table 4: Comparison of percent reduction in total microbial plaque count between the two study groups following the intervention.

	Control Group (Placebo)	Test Group (Propolis)
Mean (SD)	5.47(17.14)	11.48(11.23)
P value	0.20	

* Statistically significant at P ≤ 0.05

DISCUSSION

Preventive dental care is a key component of the management of dental caries and gingival diseases to control their underlying causes. Bacterial suppression can be an important event in preventing and arresting caries development in children who are considered at risk (23).

Currently, there is a strong trend to use natural materials as a remedy for a variety of diseases. The health field has always strived to use natural products as an alternative to the conventional allopathic formulations. Propolis is one such natural substance which has gone unnoticed in spite of its potential use in curing a large array of diseases (24).

Based on this concept, the present study was conducted to investigate the antibacterial effect of Egyptian propolis mouthwash on dental plaque in a group of children who were considered to be at high risk to caries and gingival disease due to the presence of space maintainers. A recent study by Arikan et al., (25) showed that both fixed and removable space maintainers led to an increase in the number of microorganisms in the oral cavity as well as plaque accumulation.

Data from the present study, showed no significant difference between the two groups concerning age and gender distribution, which indicates comparability of groups.

Within group comparison in the control group some reduction in the absolute bacterial count and log value has been recorded that didn't reach significant level. This reduction indicates the possible effect of dental health education and the reinforced oral hygiene instruction on patient compliance and hence plaque microbiota. This is in accordance to the conclusion of Seow et al.(26) who claimed that a single oral health education session and daily tooth brushing can lead to reduction of mutans streptococci from mouths of infected children.

In the test group both the absolute count and log value showed a highly significant drop (P≤0.0001) following intervention, which highlights the effect of propolis as an antibacterial agent.

The significant difference found in the test group supports the study of Hegde et al. who evaluated the antibacterial action of propolis on the concentration of streptococcus mutans colonizing the oral cavity of children and showed that there is a significant reduction in the number of colonies and related it to the effect of propolis extract on bacterial growth (27).

Among groups comparisons (test verses control) revealed a surprisingly significant difference between groups at baseline. This result could be attributed to the seemingly small sized sample as evident by the comparatively high standard deviation. Another factor to consider which is a limitation of study is the drop out of subjects from both groups, which compromised the randomization of subjects and probably introduced some bias. In spite of the significant difference between groups at baseline, the after intervention difference diminished which support the effect of propolis on plaque bacteria. The percent reduction in total microbial count further supports the pervious assumption.

Another variable to consider, that might have affected the result is the duration of the space maintainer in the child's mouth. Matching children with respect to this variable should have been considered.

The same trend was evident in the plaque scores, except that in the test group the mean plaque value after intervention scored significantly lower than their counterparts (P≤0.002). This contradiction could probably indicate the difference between visual plaque assessment and bacteriological estimation. The positive effect of propolis on plaque is supported by the study of Pereira et al.,(28) in which propolis mouthwash showed a significant reduction in plaque and in gingival index compared to baseline values after 45 to 90 days use. However, the study of Murray et al., (29) revealed contradictory data asserting that 10% propolis has no significant effect on dental plaque growth in spite of the slight reduction observed (14%).

Within the limitations of this study, propolis mouthwash proved to have a significant effect on supragingival plaque bacteria as well as on plaque accumulation, compared to the narrow changes recorded in the control group. The results suggest the rejection of proposed null hypothesis and support the use of propolis as an adjunctive agent in the control of dental plaque in high risk children wearing space maintainers.

CONCLUSIONS

Mouthwash containing propolis significantly reduced bacterial count and plaque accumulation when used for 3 weeks.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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