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Evaluation of The Antimicrobial Effect of Propolis Extract on Oral Microflora

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ABSTRACT

Aim: This study was conducted to evaluate the antimicrobial effect of propolis extract with different dilutions when applied as mouth wash in vivo against a known antiseptic agent (0.2%Chlorhexidine) .

Material and Methods: Ninety children were selected for this study. All children were examined clinically for dental caries and used to evaluate the antimicrobial effect of studied materials. The children were divided equally into two groups (fourty five in every group). In the first group children were instructed to rinse with 3 ml of propolis extract for specific time and divided into three sub groups while in the other group children were instructed to rinse with 3 ml of Chlorhexidine.

Result: The results of this study revealed that the use of propolis mouth wash by children achieved mean of reduction of total bacterial count. The effect of 0.2% Chlorhexidine was higher than the effect of propolis in total bacterial count reduction .

Conclusion: This study concluded that Propolis is an antimicrobial agent as it significantly reduced the total bacterial count in the saliva of children when compared by apotent antiseptic like 0.2% chlorhexidine.

INTRODUCTION

Dental caries(decay) is one of the most prevalent chronic childhood diseases worldwide and is a major problem both from a population health perspective and for individual families who have to deal with

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a young child suffering from toothache⁽¹⁾. It is characterized by colonization and accumulation of oral microorganisms on dental surfaces, resulting in the formation of dental plaque and demineralization of the tooth structure. Therefore, control of the bacterial biofilm on teeth is essential for the maintenance of oral health. Some antiseptics, such as chlorhexidine and triclosan, have been used in cariology⁽²⁾

Antiseptic mouth rinse solutions are used for improvement of the dental health (plaque and gingivitis elimination in particular) and prevention of infection caused by bacteria of the oral cavity in specific situations such as tooth extraction, intraoral surgical procedures or immune suppression due to cancer therapy or transplantation. The use of antimicrobial mouth rinses has been proposed as a means of reducing the level of oral bacteria⁽³⁾.

There are many other natural compounds like xylitol, green tea, mint and *Propolis* that have been applied as mouth rinses to reduce oral bacteria. Bees and dentists have a few things in common. They are both hardworking, industrious and have a capacity to cause a great deal of discomfort and pain. However, the power and ability to heal is also something we dentists have in common with our little winged friends too. *Propolis*, is derived from the Greek *pro* – ‘for or in defence of’ and *polis* – ‘the city’, hence, ‘defender of the city/hive’. *Propolis* is a naturally-occurring bee product. It is a hard resinous substance consisting chiefly of wax and plant extracts⁽⁴⁾

Propolis contains proteins, amino acids, vitamins, minerals, and flavonoids. Preparations with *propolis* have been found to be antimicrobial, anti-inflammatory, have antiscar effects, and to be highly antimycotic.⁽⁵⁾

MATERIALS AND METHODS

A total of ninety Egyptian children from both sexes were included in this study. Ethical approval was obtained from the Research and Ethics committee of the Faculty of Dental Medicine of

Al-Azhar University (Girls Branch), Cairo, Egypt. The study was explained to all participants' parents and they signed informed consent denoting their agreement to participate in the study. Also verbal consent from the children was obtained. All children were examined clinically for dental caries using a sterile diagnostic set including: plane mirror, sickle explorers and tweezers. The children were selected fulfilling the inclusion criteria:-

- Their age ranged from (5-12 year)
- Systemically healthy patients.
- Absence of any fixed or removable orthodontic appliances or prosthesis.
- No untreated cavities
- No history of any anti-microbial mouth wash several hours before sample collection
- No history of recent antibiotic therapy
- No change in dietary habits and day to day practices

Preparation of propolis mouth wash

Propolis extract (3ml, 5ml, and 10ml) diluted in 90 ml sterile water and placed in a hermetically sealed bottles and kept at room temperature.

Grouping of children

The children randomly distributed into two groups each of 45 (**group A and B**).

Group A (experimental group):

Forty five subjects were instructed to rinse with 3 ml of propolis extract for 5 days three times a day and divided into three sub groups;

- **Sub group1:** Children were instructed to rinse their mouth with 3ml of (10 ml of propolis extract diluted in 90 ml sterile water) for 1 minute three times a day for 5 days under their parent's supervision.

- **Sub group 2:** Children were instructed to rinse their mouth with 3ml of (5ml of propolis extract diluted in 90 ml sterile water) for 1 minute three times a day for 5 days under their parent's supervision.
- **Sub group3:** Children were instructed to rinse their mouth with 3ml of (3 ml of propolis extract diluted in 90 ml sterile water) for 1 minute three times a day for 5 days under their parent's supervision.

Group B (control group):

45 subjects were instructed to rinse with 3 ml of chlorhexidine for 1 minute ,three times for 5days under their parent's supervision.

Collection of sample

Saliva samples were collected from each child by asking him to spit in a labeled sterile container(3 ml on the average). Each subject was refrained from tooth brushing in the morning and from eating or drinking at least 1hours before sampling time

Baseline sample (S₁): The initial sample was taken before using the mouth wash by asking the child to spit in a labeled sterile container (3 ml on the average).

Second sample (S₂): The second sample was taken after using specific mouth wash for 5 days by asking the child to spit in a labeled sterile container (3 ml on the average). Procedures of sampling occurred in complete aseptic conditions because any contamination could affect the bacteriological counts

Microbiological analysis for total microbial count:

All collected saliva samples from each group were immediately submitted to the Culture and Sensitivity Unit at Regional Center for Mycology and Biotechnology at Al-azhar University.

The saliva sample(3 ml on the average) was diluted at (1:100 and 1:1000).For each dilution (20 microliters) of the sample was taken by micropipette from the sterile container.

After dilution,the sample was inoculated in plate count agar media(Also known as: Trypticase Glucose Yeast Agar; Standard Methods Agar). Each sample was cultured in triplicate. The plates were incubated at 37 C for 24 -48 hours⁽⁶⁾.

After the incubation period passed,colony forming units of saliva sample was determined by using the number of colonies in agiven dilution.

Statistical analysis

- Analysis of data was performed using SPSS 17 (Statistical Package for Scientific Studies) for Windows
- Description of quantitative variables was in the form of mean, standard deviation (SD). Data were explored for normality using Kolmogorov-Smirnov test of normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data) so parametric tests were used for the comparisons.
- Different treatments were compared using analysis of variance (ANOVA) test, followed by Tukey's post hoc test when a significant difference was detected. In each treatment, the significance of the difference between the baseline values of total bacterial count and values after treatment was calculated using Student's t test.
- Results were expressed in the form p-values that were differentiated into:
 - * Non-significant when p-value > 0.05
 - * Significant when p-value ≤ 0.05

RESULTS

1- Bacterial count (C.F.U.) at baseline

Total bacterial count was calculated as colony forming unit (C.F. U.). The mean value at baseline (before treatment) didn't significantly differ between groups. ANOVA test revealed a p value=0.965

2- Bacterial count (C.F.U.) after treatment

Total bacterial count was calculated as colony forming unit (C.F. U.). The mean value after treatment showed the lowest value in the chlorohexidine group (2405±509). The greatest value was observed in the Propolis -3ml group (1,078, 867±302,179). ANOVA test revealed a significant difference ($p < 0.00001$) between the different groups. Tukey's post hoc test revealed a significant difference between each two groups.

Table 1. Total bacterial count (C.F.U.) at baseline before treatment (S1) and significance of the difference using ANOVA test

	Test group Group A			Control group Group B
	Sub group (1) Propolis- 10ml	Sub group (2) Propolis- 5ml	Sub group (3) Propolis- 3ml	Chlorohexidine
Mean	27,680,000	28,160,000	27,400,000	26,640,000
SD	3,648,718	5,616,913	10,734,723	10,352,143
P value	0.965 ^{ns}			

ns=non-significant

Table 2. Total bacterial count (C.F.U.) after treatment (S2) and significance of the difference using ANOVA test

	Test group Group A			Control group Group B
	Sub group (1) Propolis- 10ml	Sub group (2) Propolis- 5ml	Sub group (3) Propolis- 3ml	Chlohexidine
Mean	6,753 ^a	364,000 ^b	1,078,867 ^c	2,405 ^d
SD	1,267	63,336	302,179	509
F value	186.3			
P value	<0.00001*			

* Statistically significant

Tukey's post hoc test: means with different superscript letters are significantly different

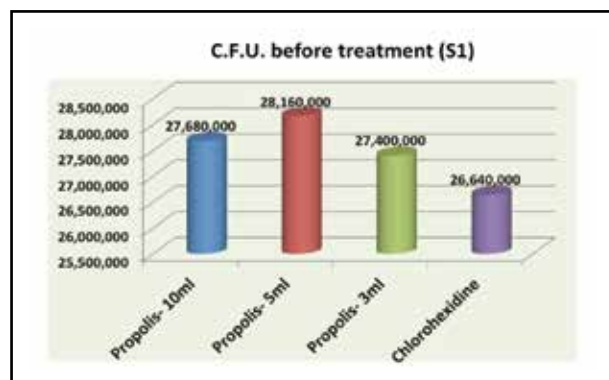


Fig. (1) Column chart showing mean total bacterial count (C.F.U.) at baseline before treatment (S1)

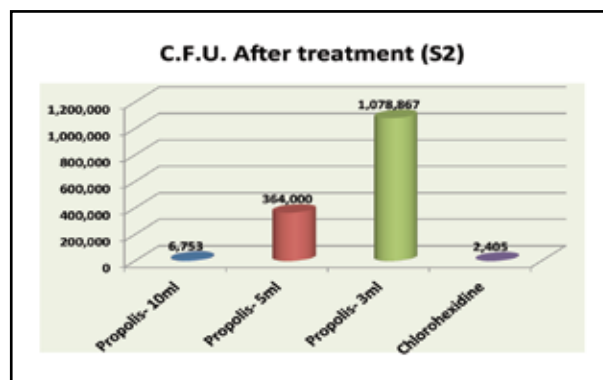


Fig. (2) Column chart showing mean total bacterial count (C.F.U.) after treatment

DISCUSSION

Oral diseases persist to be a major health problem around the world⁽⁷⁾. Apart from dental caries and periodontal diseases which are the most important global oral health problems, other conditions like oral and pharyngeal cancers and oral tissue lesions are also of important concern. Oral health is essential to general well-being and is related to the quality of life that extends beyond the functioning of the craniofacial complex. The relationship between oral diseases and activities of the microbiota of the oral cavity is well established. Over 750 species of bacteria (50% of which are still to be identified) colonize the oral cavity and a number of these are responsible in oral diseases. The world wide need for alternative treatment and preventive options and products for oral diseases that are effective, safe and economical comes from the rise in disease incidence, increased resistance by pathogenic bacteria to currently used antibiotics⁽⁸⁾.

The application of natural agents with antimicrobial activities on dental surfaces promotes a reduction in biofilm formation. In addition, these agents can inhibit bacterial colonization, growth, and metabolism⁽⁹⁾.

In this study we used propolis as it is safe and natural. It is a non toxic substance and for most people, will not cause irritation when used as supplements or applied to the skin. Propolis allergy upon ingestion seems to be less frequent than contact allergy, is probably due to its anti-allergenic and anti-inflammatory effects⁽¹⁰⁾.

In the present study the children ages (5-12) were decided as they can easily rinse their mouth without swallowing the mouthwash to avoid swallowing reflex. This is in agreement with ⁽¹¹⁾ who suggested that mouth rinses should be recommended only for those children who have demonstrated mastery of their swallowing reflex.

In this study distilled water was used for preparation of mouth rinses as alcohol used in oral care products can be irritating to the cheeks, teeth and gums. Excessive use of products that contain

alcohol may also weaken the immune system's natural ability to fight bacteria and illness⁽¹²⁾.

In our study, after the use of propolis mouth wash by children resulted in a highly significant for total bacterial count ($P < 0.05$). This agreed with a study ⁽¹³⁾ which proved that propolis had antimicrobial effect on salivary bacterial count.

The results of this study was supported by several studies ^(14,15) which studied antimicrobial effect of propolis on various oral microorganism and found that propolis had greater effect on oral bacteria and evaluated the antibacterial action of an extract of propolis on the concentration of *Streptococcus mutans* colonizing the oral cavity of young patients. They concluded that propolis extract possesses in vivo antimicrobial activity against *S. mutans* present in the oral cavity and might be used as an alternative measure to prevent dental caries

This study also agreed with previous studies that ^(4,16) who concluded that propolis is very effective on oral pathogenic microorganisms (such as *Streptococcus mutans*, *Candida albicans* and *Actinobacillus a. committans*) and concluded that propolis might be used as a natural mouthwash, an alternative to chemical mouthwashes, e.g., chlorhexidine.

In the present study, we used 3 concentration of propolis (10ml, 5ml, 3ml) diluted in 90 ml sterile water, the least mean of the total bacterial count was related to the greatest concentration (propolis 10 ml) and gradually increased in the more diluted solution. The greatest value was observed in the (propolis 3 ml) and significant difference ($p < 0.001$) between the different groups. This agreed with a study ⁽¹⁷⁾ who found that the antimicrobial activity of propolis depending on propolis sample, dosage of propolis and increased with increasing dosage without reaching the highest dosage tested.

In the present study, we used (0.02%) chlorhexidine mouth wash as control group and showed highly significant reduction ($p < 0.05$) for the total oral bacteria these results were agreed with a study ⁽¹⁸⁾ who compared the antimicrobial efficacy

of 0.2% chlorhexidine mouth rinse and mouth rinse containing 0.03% triclosan, 0.05% sodium fluoride, and 5% xylitol in reducing the streptococcus mutans count in plaque. Statistically significant reduction in the streptococcus mutans count in the plaque was seen in the control and study group from baseline level. But when both the groups were compared, the antimicrobial effect of chlorhexidine was more than the propolis group.

To conclude all our results, there was insignificant difference between the results of all tested groups before using the mouth washes (propolis and chlorhexidine) but there was significant difference ($p < 0.00001$) between these groups after using the mouth washes. The mean value after treatment showed the lowest value in the chlorhexidine group. The greatest value was observed in the propolis (3ml) group.

CONCLUSION

1. Propolis is safe and natural. It is a non toxic substance and not caused allergy upon ingestion due to its anti-allergic and anti-inflammatory effects, due to the flavonoids
2. Propolis is an antimicrobial agent as it significantly reduced the total bacterial count in the saliva of children when compared by a potent antiseptic like 0.2% chlorhexidine
3. Propolis is a subject of recent dentistry research due to its antimicrobial properties, it has been called a *natural antibiotic*

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