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EFFECT OF A PROPOLIS EXTRACT ON *STREPTOCOCCUS MUTANS* COUNTS *IN VIVO*

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ABSTRACT

Objective: To evaluate the antibacterial action of an extract of geopropolis produced by the bee Melipona compressipes fasciculata on the concentration of Streptococcus mutans colonizing the oral cavity of young patients. Forty-one young volunteers performed 21 mouth rinses divided into three rinses per day for 7 days, with no other changes in their oral hygiene and dietary habits. Saliva was collected at three time points: before the first rinse, and one hour and 7 days after the first rinse. A reduction in the concentration of S. mutans was observed in 49% of all samples collected after use of the extract, 26% showed no alterations, and an increasing in S. mutans was observed in 25%. Was performed with the Statistica for Windows 5.9 program using the Kruskal-Wallis test for analysis of variance and the Mann-Whitney U test, with the level of significance set at 5%. The 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.

Uniterms: Propolis; *Streptococcus mutans;* Dental caries; Primary prevention.

INTRODUCTION

In spite of a significant decline in some countries, such as United States, Canada, Australia and some European countries, dental caries continues to be an important public health problem in other parts of the world, as for example in Northern Brazil. Dental caries is the most prevalent disease affecting humans, and its incidence is particularly high during childhood (Araújo and Figueiredo[1], 1997).

Few microorganisms found in the oral cavity are able to adhere to the teeth and, among these, a limited group is cariogenic. The specific cariogenic microbiota consists of *Streptococcus mutans*, *Lactobacillus* and some *Actinomyces* species. However, during the initial phase of caries disease *S. mutans* is the most frequently associated microorganism (Krasse[13], 1988). In addition to its ability to adhere to teeth and survive in acid environment, *S. mutans* is transmissible, as first demonstrated by Keyes[9] (1960).

To date, prevention and control of dental caries, which include a noninvasive treatment proposal consisting of watchful waiting and monitoring, are not restricted to a single technique. In addition to traditional methods, such as periodic follow-up, brushing with fluoride-containing dentifrices, topical application of fluorides, low-sucrose diet, sealant placement and at-home rinsing with fluoride-containing solutions, some cases require a more effective control (Maltz[14], 1996). It is therefore understandable that researchers are currently interested in the promising perspectives that natural substances offer as alternatives for the control of caries disease in terms of antimicrobial response and lower associated risks. Based on literature reports showing that propolis resin is a product with antiinflammatory and bactericidal activity, several *in vitro* and some *in vivo* studies have demonstrated its potential use in the treatment of bacterial diseases (Bianchini and Bedendo[3], 1988, Fernandes Jr., et al.[6] 2001, Koo, et al.[12], 2002, Manara, et al.[15], 1999, Silva[23], 2000).

The purpose of the present study was to evaluate the *in vivo* antimicrobial activity of an extract prepared with propolis produced by *Melipona compressipes fasciculata* bees (Ministry of Agriculture, registration number 0005/731) and used as mouthrinse on the concentration of *S. mutans* present in the oral cavity of young individuals.

MATERIAL AND METHODS

This study enrolled 41 young volunteers of both sexes, including high-school and university students, ranging in age from 11 to 30 years. The patients were assigned to three groups according to the place of referral: group A – public school (n=11), group B – private dental school (n=14), and group C – private practice (n=16). Samples of whole saliva were collected in the morning, 2 hours after the first meal of the day. The first collections were stimulated with paraffin-based chewing gum (1 minute), collected into sterile flasks (3 mL on the average) and immediately seeded in the laboratory. The students were then asked to rinse their mouth with 3 mL of the propolis extract solution during 1 minute and a second saliva sample was collected 1 hour later in the same way as described for the first sample. A third saliva sample was obtained after 7 days. The volunteers performed 21 mouth rinses divided into 3 rinses/day for 7 days, with no other changes in their oral hygiene and dietary habits. A total of 123 samples were obtained

The microbiological analyses were carried out using the Caritest-SM system (Technew, Rio de Janeiro, RJ, Brazil) according to manufacturer recommendations. This culture medium is selective for *S. mutans* and permits semiquantitative analysis of this microorganism in salivary samples. Gram staining, catalase test and mannitol fermentation were used for the presumptive identification of some bacterial isolates (Konemen[11], 1997).

Statistical analysis was performed with the software Statistica for Windows 5.9 program using the Kruskal-Wallis test for analysis of variance and the Mann-Whitney U test, with the level of significance set at 5%.

RESULTS

Of the 123 saliva samples collected from the 41 volunteers, those from 21 volunteers presented bacterial counts ranging from 1250 to 1,000,000 CFU/mL saliva, whereas no bacterial growth was observed in 20 samples before or after the use of the mouthrinse (Table 1).

Table 2 displays the differences in microbial growth between the first and second collection (no extract use and 1 h after the first rinse) and between the first and third collection (no extract use and after 7 days). These differences are reported as base 10 logarithms and are presented by the letters I (increase in the number of colonies), D (decrease in the number of colonies) and NC (no change). Samples showing no bacterial growth at any time were excluded.

Based on the data in Table 2, the relative frequency of samples presenting an increase, decrease or no change in the number of colonies between collections 1 and 2 and 1 and 3 was calculated.

Among samples showing growth of *S. mutans* between collections 1 and 2, a decrease in the number of colonies was observed in 62%, an increase in 14% and no change in 24%. Comparison between collections 1 and 3 revealed a reduction in bacterial concentration in 81% of the samples, an increase in 9.5% and no change in 9.5%.

Statistical analysis of the results by the Student t-test showed a significant difference in the number of *S. mutans* between collections 1 and 2 (mean ± SD, 4.275 ± 0.8459 versus 2.809 ± 2.0911; t = 2.8783 and p = 0.0031), and between collections 1 and 3 (4.275 ± 0.8459 versus 2.4184 ± 1.9999; t = 4.8589 and p<0.0001). These results indicate a reduction in the number of *S. mutans*, i.e., an effect of propolis on bacterial growth both after the beginning (collection 2) and at the end of treatment (collection 3).

Analysis of variance to determine the relationship between the number of mouth rinses and bacterial counts indicated a significant difference.

TABLE 1- Number of *Streptococcus mutans* (CFU/ml) in each saliva sample

VOLUNTEERS	1st SAMPLE
1st	NG
2nd	NG
3rd	10.000
4th	NG
5th	NG
6th	50.000
7th	2.500
8th	250.000
9th	NG
10th	5.000
11th	NG
12th	50.000
13th	NG
14th	5.000
15th	NG
16th	NG
17th	10.000
18th	NG
19th	NG
20th	NG
21st	NG
22nd	NG
23rd	1250
24th	NG
25th	1250
26th	NG
27th	1.000.000
28th	1250
29th	5.000
30th	NG
31st	10.000
32nd	50.000
33rd	10.000
34th	NG
35th	250.000
36th	NG
37th	250.000
38th	100.000
39th	10.000
40th	NG
41st	50.000

* NG = no growth.

DISCUSSION

Within the philosophy of health promotion, the extract of propolis produced by the Tiúba bee (*Mellipona compressipes*) may represent a new option, with this substance being easily obtained and of low cost and showing long-term beneficial effects (Oliveira[16], 2004).

The inhibitory effect of the extract could not be evaluated in 20 samples due to the lack of bacterial growth after the first collection.

In view of the diversity in the counting results, some important facts were addressed. For example, between the first and second collections, 13 samples (62%) presented a decrease in the number of colonies; samples 3, 10 and 28 (14%) showed an increase in the number of colonies; and no change was observed for samples 6, 35, 38, 39 and 41 (24%) (Table 2). A significant reduction in the number of colonies in the samples is the result of the effect of the propolis extract on bacterial growth. Samples that showed an increase or no change in the number of bacteria between collections 1 and 2 might have been influenced by overlapping factors, such as a delayed peak formation of colonies, i.e., more than 2 hours after the meal, together with the short period for an effective action and the transmissible nature of *S. mutans* (Dutra, et al.[5], 1997, Kohler[10], 1984, Ikeno, et al.[8], 1991, Figueiredo and Faster[7], 1997, Ota, et al.[17], 1998). In addition to these hypotheses, one should take into account that caries is a mixed infection and the oral microflora is highly diverse. Thus, a single bacterial species such as *S. mutans* can be present in the mouth in three different serotypes, i.e., serotypes C, E and F (Beihton, et al.[2], 1991, Dasanayake, et al.[4] 1995, Sarni, et al.[21], 1998, Zárate[24], 1999, Shibata, et al.[22], 2003). Ray, et al.[20] (1999) also demonstrated that host binding characteristics are as important as the characteristics of bacterial adhesion in the process of colonization. The authors suggested that salivary amylase may show the best binding to *S. mutans*. Furthermore, Palmer, et al.[18] (2003) raised the possibility that bacterial interactions have a key role on the importance for colonization, i.e., the authors investigated whether bacteria such as *S. mutans* require the presence of other bacteria such as *Actinomyces* for colonization.

The results of the first and third collections, corresponding to the moments before and after the 7-day exposure to the extract, showed a reduction in the number of colonies in most samples (81%), whereas the number of bacteria increased (9.5%) or remained unchanged (9.5%) in a small part of the samples, findings that require some considerations regarding the quality of the resin and its properties. Park, et al.[19] (1997) demonstrated various levels of action for propolis derived from different regions. However, Fernandes Jr., et al.[6] (2001) stated that the action

TABLE 2- Logarithmic difference in the number of *Streptococcus mutans* (CFU/ml + 1) between collections 1 and 2 and between collections 1 and 3

VOLUNTEERS	Log 1	Log 2	Log 3	DIF 1-2	Log 1-2	DIF 1-3	Log 1-3
3 nd	4.000	4.699	4.699	I	-0.699	I	-0.699
6 nd	4.699	4.699	0.000	NC	0.000	R	4.699
7 nd	3.398	0.000	0.000	R	3.398	R	3.398
8 nd	5.398	4.000	5.398	R	1.398	NC	0.000
10 nd	3.699	4.000	4.000	I	-0.301	I	-0.301
12 nd	4.699	4.000	0.000	R	0.699	R	4.699
14 nd	3.699	0.000	0.000	R	3.699	R	3.699
17 nd	4.000	3.699	3.398	R	0.301	R	0.602
23 nd	3.097	0.000	0.000	R	3.097	R	3.097
25 nd	3.097	0.000	0.000	R	3.097	R	3.097
27 nd	6.000	4.000	4.000	R	2.000	R	2.000
28 nd	3.097	4.000	3.097	I	-0.903	NC	0.000
29 nd	3.699	3.398	3.398	R	0.301	R	0.301
31 nd	4.000	0.000	3.699	R	4.000	R	0.301
32 nd	4.699	3.398	3.398	R	1.301	R	1.301
33 nd	4.000	0.000	0.000	R	4.000	R	4.000
35 nd	5.398	5.398	4.000	NC	0.000	R	1.398
37 nd	5.398	0.000	3.699	R	5.398	R	1.699
38 nd	5.000	5.000	4.000	NC	0.000	R	1.000
39 nd	4.000	4.000	0.000	NC	0.000	R	4.000
41 nd	4.699	4.699	4.000	NC	0.000	R	0.699

* NC = no change, R = reduced, I = increased.

of propolis depends on the type of infection installed and indicated that sting less bees are producers of a propolis resin with high germicidal activity.

CONCLUSION

It may be concluded that the propolis extract tested possesses antimicrobial activity against *S. mutans* present in the oral cavity. The extract might be used as an alternative measure to prevent dental caries.

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