INFLUENCE OF PROPOLIS ON CARIES - AN *IN VIVO* STUDY IN RATS

INFLUÊNCIA DA PRÓPOLIS SOBRE CÁRIE - ESTUDO IN VIVO EM RATOS

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ABSTRACT: Propolis is well known by its antibacterial and anti-inflammatory properties. The aim of this study was to evaluate the influence of a hydro-alcoholic suspension of propolis daily consumed on caries using an infected rats model. With this purpose, Wistar rats were infected with a suspension of *S. mutans* (CCT1901) strain cells previously to the experiment baseline. Then the animals were divided in two groups: Control group (n=10) which were fed with a cariogenic diet (sucrose 56% and powdered skim milk 28%) and drinking water *ad libitum*. Experimental Group (n=10) were fed with the same diet and water containing propolis (1mg/ml). After 90 days, the animals were sacrificed and the molar teeth caries lesions were registered and analyzed (Chi-square test, α=0,05). Even though the number of caries lesions did not vary between groups, no lesions with score 3 (partial or total coronal destruction) in experimental group were observed, against 12 observed in control group. Although propolis did not avoid caries, it has influenced on caries process since the number of teeth with score 3 were not observed in experimental group which received propolis in daily diet.

KEYWORDS: Propolis. Caries. Rats. *Streptococcus mutans*.

RESUMO: *A própolis é conhecida popularmente pelas suas propriedades antibacterianas e antiinflamatórias. Neste trabalho analisamos a influência do consumo de um extrato hidro-alcoólico de própolis sobre a cárie em modelo animal. Com esta finalidade, ratos Wistar foram infectados com a cepa de S. mutans (CCT1901) previamente ao início dos experimentos. Os ratos foram divididos em dois grupos: Grupo controle (n=10): alimentados com dieta cariogênica (contendo 56% de sacarose e 28% de leite em pó) e água ad libitum. Grupo experimental (n=10): a mesma dieta cariogênica e a água de beber foram acrescidos de extrato alcoólico de própolis (1mg/ml). Após 90 dias, os animais foram sacrificados e as lesões de cárie em molares foram analisadas quanto ao número de lesões e quanto ao grau de severidade (Índice de Mellamby). Os resultados foram submetidos ao teste Qui-quadrado (α=0,05). O número de lesões cariosas foi semelhante entre os grupos. Foi observado um número maior de lesões grau 3 (destruição parcial ou total da coroa dental) no grupo controle (n=12), por outro lado, o grupo experimental não apresentou nenhuma lesão com este grau de severidade. Concluímos que embora a própolis não tenha evitado o aparecimento da cárie, foi observada diferença estatística quanto ao grau de severidade; sendo esta menor no grupo experimental que recebeu própolis na sua dieta diária.*

PALAVRAS-CHAVE: *Própolis. Cárie. Ratos. Streptococcus mutans.*

INTRODUCTION

Nowadays, science is looking for alternative medicine in nature, which cause less side effects on the human body. Among these substances propolis is in evidence, a resinous wax-like substance which bees collect from plants and buds used as glue or putty to line their hives and fill up cracks.[1] Propolis is known for its medicinal properties such as bone tissue regeneration[2], immunological properties[3-5], antibacterial[6-12], and antifungal activities on yeasts of *Candida* genera[12,13].

In dentistry, its properties have been studied for many purposes. Scheller et al.[3] studied the pulp regeneration of teeth treated with propolis; Hernandes & Hernandes[14] observed satisfactory results by applying propolis on buccal ulcers; Silveira et al.[15] observed that patients with gingivitis that received applications of medicine with propolis had a faster regression of painful symptoms. In 2003, Gebara et al.[16] evaluated the effect of subgingival irrigation with a propolis extract and concluded that the subgingival irrigation as an adjuvant to periodontal treatment was more effective than conventional treatment *per se*.

The antimicrobial properties of propolis have been attributed to flavonoids, phenolic acids, diterpenic acids, flavonoid aglycones (flavones and flavanones) and coumarins.[8,9] According to Takaisi-Kikuni & Schilder[17] propolis inhibits

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bacterial growth by preventing cell division and disorganizing the cytoplasm, the cytoplasmic membrane and the cell wall, causing a partial bacteriolysis, and inhibiting protein synthesis. Nishio et al.[18] studied an ethanolic extract of Brazilian propolis and observed its antibacterial activity against 7 strains of *Streptococcus mutans*.

Ikeno et al.[19] observed that rats which received propolis in their diet had less caries than the control group fed only with cariogenic diet. There are studies which found cariostatic activities in desalivated rats model using ethanolic extracts of total propolis and some of its components.[20,21] Considering the aforementioned properties of propolis, we decided to study the influence of a hydro-alcoholic solution of propolis on caries using *S. mutans* infected rats model.

MATERIAL AND METHODS

Propolis extract: Propolis was collected from the Vale do Paraiba region, in São Paulo State located in the Southeast of Brazil. To obtain the alcoholic extract, 450g of *in natura* propolis were suspended in 1.500 mL of ethanol (Merck). This suspension was kept away from light and was shaken every day for 15 minutes for a period of 30 days. After this period, it was decanted for about 48 hours and subsequently filtered. The alcohol was partially evaporated in order to permit the lyophilisation process and it was stored in a freezer (-20° C) until use. For the experiments, the lyophilized propolis was weighed, dissolved in ethanol (99.5%) and then added to drinking water.

Animals: 20 male rats (*Rattus novergicus,* Wistar) were used. Since 17th day old until 21st day old, streptomycin (2mg/mL) was given in drinking water of animals to depress the oral flora. The experimental diet described below was given to the litters since 20th day old. At the age of 20 days, the animals were inoculated with 0.2mL of 10^8 CFU/mL suspension of *S.mutans* (CCT1901), a streptomycin resistant strain, two days before the diet experiment had already started. The success of inoculation was checked on the first day (litters are 22 days old) of the experiment cultivating material collected by oral swabbing.[22,23] In summary the rats treatment before starting the experiment followed the scheme below (Figure 1):

Figure 1 – Scheme of rats treatment before starting the experiment.

Age Event	17th day	18th day	19th day	20th day	21st day	22nd day
Streptomycin						
Cariogenic diet						
Inoculation of bacteria						
Starting experiment						

At the 23rd day old, the animals were divided in two groups:

Control group: The animals were fed with high cariogenic diet (containing 56% of sucrose and 28% of skin milk powder (Molico-Nestlé) and, sterile distilled water *ad libitum.*[24]

Experimental Group: The animals were fed with the same diet described above and propolis was added to the drinking water (1mg/mL). All the bottles of this group were covered with aluminium foil to avoid the light exposure.

Both groups received the diet for 90 days, the food was moistened with sterile distilled water before feeding the animals. The food and water of all animals were replaced daily. At the 90th day, the animals were killed and their jaws were removed and fixed in a 10% formaldehyde solution for 48 hours. After this period, the material was dissected and then dyed with 0.5% fucsin for 4 hours.[19]

The jaws were sectioned using a diamond impregnated disc (KG-Sorensen). The section was made from the mesial to the distal area, to study the depth of the caries lesion.

The material was evaluated according to the degree of severity of the lesions. Although both halves were evaluated, only one score was attributed for each tooth, as preconized by Mellanby's Index[25] (Table 1).

Table 1: Mellanby's Index[25] used to score caries lesion progression stage.

Score	Degree of caries
0	Absence of lesion
1	Enamel lesion
2	Dentin lesion
3	Partial or total coronal destruction

Statistical analysis: The Chi-square test was used for two independent samples with α=0.05. The Standardized Residual was used to observe which proportion was the major contributor to the significant difference found with Chi-square test.

RESULTS

During the experiment no differences in the drinking habits of the animals were observed, both groups consumed about the same volume of water. Each animal from the experimental group consumed about 50mg of propolis per day.

After 90 days, the animals were sacrificed and the teeth were examined. The results are shown in Table 2.

Table 2: Distribution of each severity degree among molar teeth of rats.

SEVERITY DEGREE	FREQUENCY OF EACH DEGREE			
	Control Group		Experimental Group	
	Maxillary teeth	Mandibular teeth	Maxillary teeth	Mandibular teeth
0	31	11	34	14
1	21	9	15	12
2	8	28	11	34
3	0	12	0	0
Total	60	60	60	60

It was demonstrated that the maxillary teeth did not show any difference between experimental and control groups (Chi-square test; α=0,05).

The results of the mandibular teeth of both groups were analyzed using the same test and we could verify that the groups were different (Chi-square test;α=0,05). It is noteworthy that we did not find score 3 lesions in this group (Table 2), the Standardized Residual values revealed that the major contributors for this difference were the teeth with score 3.

Table 3: Distribution of each severity degree in molar teeth of rats.

SCORE	FREQUENCY EXPERIMENTAL GROUP	FREQUENCY EXPERIMENTAL GROUP	STANDARDIZED RESIDUAL
0	14	11	+0.90
1	12	9	+1.00
2	34	28	+1.30
3	0	12	-3.46

DISCUSSION

Propolis has attracted a lot of attention in recent years as a useful substance applied in medicine and cosmetics, even it has been known in folk medicine since ancient times. The antibacterial, antiviral, antifungal and antiprotozoan properties of propolis has been widely studied.[26]

In previous studies we and others research groups demonstrated the antibacterial[10,11,19,27,28] and antifungal activities[12,13] on strains isolated from oral cavity. *Streptococcus mutans* called our attention by its sensitivity to propolis;[10,11,28] since from MS group it is the species most associated with human dental decay.[30] So our hypothesis is that propolis extract could have some influence on caries process. Thus, a *Streptococcus mutans* infected rats model was proposed.

In previous experiments, we could not achieve any high degree of severity lesion with a experimental period of 42[20]to 56 days[23], even in animals from the control group which received only the cariogenic diet. So, the experimental period was extended to 90 days (data not shown). These differences could be explained by some reasons: a) it was used a *Streptococcus mutans* strain instead *Streptococcus sobrinus* as used by Ikeno et al.,[19] Koo et al.,[20] and Koo et al.[21] ; b) it was demonstrated by Soet et al.[30] that for rats *S. sobrinus* is more cariogenic than *S. mutans*, then the caries lesion could appear earlier in experiments with *S. sobrinus*; and c) it was not used desalivated rats as used by Koo et al.[21] and Koo et al.[20], this procedure cause a low saliva flow causing an increase of smooth surface caries, because a highly acidogenic flora was selected.

Concerning to the number of lesions it was observed almost the same results for the experimental and control groups but the severity degree was different. Ikeno et al.[19] found different results by analyzing only the dentin caries of the first and second molar teeth. Using the same method of evaluation, we observed 37% of reduction in number of caries in the experimental group (data not shown). The different bacterial species used in our experiments could justify this variation. For animals treated with propolis it was found only dentin and enamel lesions without total or partial coronal destruction, on the other hand, animals treated only with cariogenic diet (control group) had lesions with a higher level of severity.

Besides, after analyzing the quality of lesions, also considering the enamel lesions (Table 2), the data showed that there was an inhibition of the caries process, even though it did not prevent them. These data are in accordance with Koo et al.[20] that found either a cariostatic effect of a Brazilian propolis even with a desalivated rat infected with *S. sobrinus* model was used. Koo et al.[21] studied two substances, specifically: tt-farnesol and apigenin; common members of propolis composition and it was demonstrated that both have cariostatic activity but, substances like fluoride, chlorhexidine are still more efficient.

Nishio et al.[18] showed that propolis could be used to control the caries process, the authors isolated three cinnamic acids from propolis and verified that two of them strongly inhibit acid formation from sucrose by *S.mutans* and, the third inhibit the synthesis of insoluble glucans by glucosyltransferase. It is known that these insoluble glucans contribute to the pathogenic potential of *S. mutans* and *S. sobrinus*.[31] According to Ikeno et al.[19], propolis has antimicrobial activity and clearly inhibits insoluble glucans synthesis and partially inhibits glycosiltransferase activity in some species of MS group: *S. mutans, S. sobrinus* and *S. cricetus* .

Zárate Pereira [32] found interesting results dropping propolis solution every day on the surface of human teeth enamel, with this procedure the enamel becomes more resistant when

submitted to the Knoop hardness test. They do not explain what substance of propolis composition is responsible for this property. Gimalia et al.[33] also studied this property by immersing enamel slices in different concentration solutions of propolis. The Vickers hardness values increased accompanied by higher concentrations of propolis in the solution.

In summary, our results show that propolis possess a cariostatic activity in a *S. mutans* infected rat model which is the most common species isolated from human decay. Probably, the cariostatic activity of propolis is due to a group of properties that is achieved by the large variety of substances found in its composition. Properties like: antibacterial activity, its influence on glycosiltranferase and on the resistance of human enamel and others. Much more investigations are need to establish which are the substances and the mechanisms and if they would work alone or together leading to this cariostatic activity. But there is no doubt that this material is a source for a variety of substances that should be studied aiming its use in the clinical dentistry routine, preventing not only caries but also other oral pathologies.

ACKNOWLEDGEMENT: This research was supported by FAPESP (Grant 94/4865-3).

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