EVALUATION OF THE IN VITRO ANTIMICROBIAL ACTIVITY OF ALCOHOLIC SOLUTION AND AQUEOUS EXTRACT OF BEE PROPOLIS AGAINST *ENTEROCOCCUS FAECALIS*

ABSTRACT

AIM: The purpose of this study was to analyze the in vitro antimicrobial activity of aqueous and alcoholic extracts of propolis at 1% and 3% concentrations against Enterococcus faecalis (ATCC® 19433). **MATERIAL AND METHODS:** Initially, the microbial suspension was seeded in a Brain Heart Infusion Agar (BHIA) culture medium, distributed in 20 Petri dishes. Then, 4 soaked filter paper discs were placed on the surface of the inoculated medium of each plate for 1 minute in 1 mL of the following substances: C+ (positive control, n=20): 2% chlorhexidine gel; C- (negative control, n=20): saline solution; S1 (n=10): 1% bee propolis alcoholic solution; S2 (n=10): 3% bee propolis alcoholic solution; E1 (n=10): 1% aqueous propolis extract; E2 (n=10): 3% aqueous propolis extract. One filter paper disc of each (C+, C-, S1 and S2) was placed in a set of 10 Petri dishes, whereas one filter paper disc of each (C+, C-, E1 and E2) was placed in the other set of 10 Petri dishes. **RESULTS:** The results obtained after incubation at 37°C for 24 hours under microaerobic conditions revealed that S2 showed higher mean levels of microbial growth inhibition as compared to E1, E2 and S1. There were no significantly statically differences between the groups, except for the S2 group and C- group. Mean levels in all other groups were lower than in the C+ group. **CONCLUSION:** The study concluded that 1% and 3% bee propolis alcoholic solution had lower antibacterial activity against Enterococcus faecalis as compared to 2% chlorhexidine gel.

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KEYWORDS

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INTRODUCTION

Root canal infections cause apical periodontitis. One of the greatest challenges of Endodontics is to treat such a pathology. Studies have shown that solving the problem consists of controlling the microorganisms that are active in the process[1,2]. For this goal to be achieved, the different stages of treatment should identify techniques and chemicals that act in the endodontic microflora.

Modern endodontic techniques aimed at eliminating microorganisms of the root canal system where the pulp necrosis occurred. In clinical situations requiring the use of intracanal medication, calcium hydroxide has been widely used due to its biological and microbiological properties. Sometimes it has proven ineffective in the destruction of some microorganisms because of the difficulty for the medication to reach the site of action and the buffering capacity of hydroxyapatite3. Additionally, scientific evidence has pointed to the resistance of certain microorganisms to calcium hydroxide[4,5].

The high prevalence of *Enterococcus faecalis* in endodontic post treatment complications is attributed, in particular, to its ability to adhere to collagen, strengthened by the presence of human serum and, in the colonization process, invade the dentinal tubules; interfere with host defenses; resist to the action of antimicrobial substances, hindering the endodontic treatment; and become a constant source of reinfection of the obturated canal[1,6-8].

Bee propolis is a resinous mixture varying in color and consistency that honey bees collect from tree buds, sap flows, or other botanical sources[9]. Currently, different chemical structures are identified in propolis, such as those belonging to the following classes: alcohols, aldehydes, aliphatic acids, aliphatic esters, amino acids, aromatic acids, aromatic esters, flavonoids, hydrocarbon ester, ether, fatty acids, ketones, terpenes, and sugars[10].

Some authors have suggested the use of propolis as intracanal medication during endodontic therapy. Studies have shown favorable results regarding biocompatibility and cytotoxicity of this substance[11-13].

With regard to antimicrobial activity, some studies have been conducted in order to investigate propolis activity in varied concentrations against different microorganisms[14-20].

Faced with the need for intracanal medications that are effective against microorganisms commonly found in endodontic infections, the purpose of this study was to evaluate the in vitro antimicrobial activity of aqueous and alcoholic extracts of propolis at 1% and 3% concentrations against *Enterococcus faecalis*.

MATERIAL AND METHODS

Aqueous extract of propolis was obtained in the state of Minas Gerais, and the alcoholic solution was from the state of Rio Grande do Sul. Prior to the study, the propolis samples were analyzed at the Center for the Study of Social Insects of the Institute of Biosciences of Rio Claro. To obtain the samples used in this study, the aqueous extract and the alcoholic solution of propolis were diluted in 80% ethanol, yielding 1% and 3% aqueous extract, as well as 1% and 3% alcoholic solution of propolis.

We prepared 1000 mL of Brain Heart Infusion Agar (BHIA, Becton Dickinson and Co., Cockeysville, MD) culture medium using deionized water as the solvent. Next, the prepared medium was sterilized, and after cooling to 50°C temperature, it was placed into 20 sterilized Petri dishes (150x10mm). Each dish received 50mL of culture medium, reaching a thickness of 4mm. Then, the dishes were taken to a laminar flow hood (Veco, São Paulo, Brazil) at room temperature until complete cooling of the culture medium.

Enterococcus faecalis obtained from the standard strain (ATCC - 19433) was seeded on the surface of the agar medium, which was distributed in the other two Petri dishes. Then, they were taken to a culture oven (Model 002 CB Sanem Ltd, São Paulo, Brazil) to be incubated for 24 hours at 37°C.

After this period, bacterial suspensions adjusted to matching the turbidity of McFarland's standard scale number 1 were obtained by diluting the bacterial colonies grown in BHIA in distilled water.

Next, an aliquot of 1.0 mL of microbial suspension was placed in each of the 20 Petri dishes containing the culture medium. The suspension was seeded on the entire surface of the medium using a swab to obtain a confluent growth.

On the surface of the inoculated culture of each of the 20 dishes, we placed 4 discs of moistened filter paper of one centimeter in diameter (UPF, Passo Fundo, Brazil) previously prepared and sterilized in the Pathology Laboratory of the University of Passo Fundo. Before being placed on the surface of the culture medium, the discs were soaked for 1 minute in 1 mL of the substances under investigation: C+ (positive control, n = 20): 2% chlorhexidine gel; C- (negative control, n = 20): saline solution; S1 (n = 10): 1% alcoholic solution of propolis; S2 (n = 10): 3% alcoholic solution of propolis; E1 (n = 10): 1% aqueous propolis extract; and E2 (n = 10): 3% aqueous propolis extract. One filter paper disc of each (C+, C-, S1 and S2) was placed in a set of 10 Petri dishes, where as one filter paper disc of each (C+, C-, E1 and E2) was placed in the other set of 10 Petri dishes.

Then, the Petri dishes containing the inoculated medium and the discs sprayed with

test solutions were pre-incubated at room temperature for 1 hour to allow for the diffusion of substances prior to microbial growth. Next, they were incubated at 37ºC for 24 h in a microaerophilic environment in a candle jar.

After the incubation period, the diameter of the zones of inhibition of bacterial growth around each disc was measured by using a ruler and a reflected light source. Two measurements were made perpendicularly to each other, and the average was used for analysis.

The Kruskal-Wallis test was used for comparison between the groups, and the Student–Newman Keuls *post hoc* test for mean differences.

RESULTS

Table 1 shows the halos of inhibition of bacterial growth in descending order of mean scores. The measurements showed the following results: S2>S1=E2=E1. All experimental groups, as well as the negative control group, had a lower halo of inhibition of microbial growth as compared to the positive control group (2% chlorhexidine gel). The S1, E1 and E2 groups did not differ significantly from the negative control. The S2 group showed greater halo of inhibition as compared to the negative control, which was a statistically significant difference.

DISCUSSION

Significant research efforts have been put forward in Endodontics to treat infected root canals, reduce the microbial population, and promote sanitation of root canal systems.

Sodium hypochlorite and chlorhexidine used during biomechanical preparation, and the different calcium hydroxide pastes are examples of substances that have overcome time constraints and are still used to fight against endodontic infections. Each one has its own importance. However, the search for an ideal substance that could achieve greater effectiveness in eliminating endodontic microorganisms, while presenting biocompatibility and avoiding cytotoxicity, will drive scientific research.

Enterococcus faecalis was chosen because it is a microorganism that is part of the endodontic microbiota, mainly related to cases of endodontic failures, in which lesions refractory to treatment do not repair through conventional endodontic therapy. This Gram+ anaerobic microorganism is able to adapt to high pH levels (above 12), tolerating the mechanism of action of calcium hydroxide even when in direct contact[1,6,7].

The diffusion test on solid medium is an established method for assessing the antibacterial activity of a particular substance. However, the limitations that it presents should also be considered. Among them, the inability to provide equal conditions to

compare substances with different solubility and diffusivity could be mentioned.

Furthermore, the presence of bacterial enzymes, medium composition, inoculum density, incubation time, temperature, and medication stability are all factors that could cause conflicting results.

Table 1. Mean and standard deviation of the halos of inhibition of bacterial growth in the experimental and control groups.

	Experimental groups				Control groups	
	S1 (n=10)	S2 (n=10)	E1 (n=10)	E2 (n=10)	C+ (n=20)	C- (n=20)
Mean (mm)	2.00±3.23[a]	6.90±2.60[b]	1.40±2.95[a]	4.00±4.32[a]	20.10±2.48[c]	0±0[a]

* Groups followed by the same letter do not differ statistically (α=5%).

When using this methodology, the low antibacterial activity should be taken into account, given that it may be related to substance diffusion difficulty. In this study, we used both the aqueous extract and the alcoholic solution of propolis, given that agar diffusion tests are more efficient for water-soluble substances as compared to other materials. Because they are water-soluble substances, one could infer that using the agar diffusion test is not appropriate to justify the low scores of microbial growth inhibition halos in the experimental groups.

The results for 1% and 3% aqueous extract and 1% alcoholic solution of propolis showed mean values of 1.4, 4.0 and 2.0 mm of inhibition halos, respectively. They exerted a low action on *Enterococcus faecalis*, which was not statistically different from the negative control. This fact allows for questioning the use of these substances in clinical situations. If their action was not effective in direct contact with the microorganisms, it is hard to believe that they will behave differently when the microorganism is inside the root canal.

A comparison between the results obtained for 3% alcoholic solution and aqueous extract of propolis produced encouraging results, with mean halo inhibition of 6.9 mm. However, a simple comparison between this result and the mean scores of 2% chlorhexidine gel group revealed that the mean score obtained for the group of 3% alcoholic solution was less than 50% of the mean score of the chlorhexidine group. Although it was possible to detect that 3% alcoholic solution presented better results than its propolis analogues, this finding must be carefully interpreted.

We were not able to compare our findings to those of other studies. The study by Groisman et al.[15] (2005) comparing propolis solution and its association with chlorhexidine has used other microorganisms and engaged a different method.

The findings of this study can be interpreted and used as a principle for the construction of a scientific rationale for the use of propolis in endodontic therapy. The fact that only a monoculture was used does not allow for the extrapolation of results to situations where mixed cultures are present. However, this research can serve as a basis for the development of further studies that contribute to the use of propolis as an intracanal medication.

CONCLUSION

Based on the results of this study, we can conclude that: (1) the alcoholic solution of propolis at 3% concentration showed a higher antibacterial activity than 1% and 3% aqueous extracts and 1% alcoholic solution of propolis; (2) the alcoholic solution and aqueous extract of propolis at 1% and 3% concentrations showed lower antibacterial activity against *Enterococcus faecalis* as compared to 2% chlorhexidine gel.

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