Propolis protects against oxidative stress in human saliva

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Abstract

Oxidative stress occurs as a consequence of imbalance between free radicals and their inactivation by antioxidant defense system, and it is capable of causing damage to various cellular and extracellular constituents in the oral cavity. Therefore, the aim of this study was to determine the effect of *Apis mellifera* propolis (AMP) and *Melipona favosa* propolis (MFP) on the Fenton reagent-induced oxidative stress of human saliva. Human saliva was incubated for 10 min at 37°C in the presence of Fenton reagent (combination of 35% hydrogen peroxide and 1 mM iron sulfate), to induce the oxidative stress, and propolis ethanolic extract dilutions. After the incubation, the salivary TAA was assayed by the ABTS method. It was found that the Fenton reagent caused a decrease in salivary TAA. AMP dilution protected and even increased the salivary TAA after the Fenton reagent-induced oxidative stress. MFP only protected the salivary TAA against oxidative stress. In conclusion, propolis could be used to maintain and even increase the antioxidant capacity of saliva during an oxidative stress.

Introduction

Saliva is the first biological fluid that encounters the possible free radicals found in the consumed food. The salivary antioxidant system includes various molecules, the most important of which are uric acid and the peroxidase systems (Greabu et al., 2007). Uric acid contributes to approximately 70% of the total salivary capacity (Nagler et al., 2004). Despite increasing use of propolis worldwide (Marcucci, 1995; Cheng and Wong, 1996), few studies have been carried out to determine the effect of propolis on saliva antioxidant activity.

Oxidative stress, occurring as a consequence of imbalance between free radicals and inactivation of these species by antioxidant defense system, is capable of causing damage to various cellular and extracellular constituents in the oral cavity. Therefore, the development of new therapies for the treatment of the oral cavity diseases is of great relevance (Walker, 1996). In Dentistry, the use of propolis has been proposed in different areas including cariology (Ikeno et al., 1991; Park et al., 1998), oral surgery (Magro-Filho and Carvalho, 1990, 1994), endodontics (Al-Shaher et al., 2004; Silva et al., 2004), oral pathology (Silva et al., 2000), periodontology (Gebara et al., 1996; Murray et al.,1997), and dental traumatology (Martin and Pileggi, 2004). However, it is not clear the effect of propolis on the antioxidant status of human saliva. Therefore, the aim of this study was to determine the effect of both *Apis mellifera* propolis and *Melipona favosa* propolis on the Fenton reagent-induced oxidative stress of human saliva.

Materials and Methods

Propolis sample

Apis mellifera ethanolic propolis extract (20%, w/v) was provided by La Casita de la Miel (Maya, Mérida, Venezuela). The *Melipona favosa* ethanolic propolis extract was obtained by extraction of 30 g of propolis in 100 mL of 95% ethanol. The 1/10 dilutions of both extracts were used in the treatment of human saliva.

Saliva sample

Whole saliva was collected in a quiet room between 9 and noon to avoid circadian changes, and was obtained by expectorating into disposable tubes. About 1 mL of whole saliva was collected in tubes and centrifuged immediately to remove any cell debris (5,000 rpm for 5 min). The supernatant was removed and used for the determination of TAA.

Saliva treatment

Saliva sample was incubated for 10 min at 37°C in the presence of Fenton reagent (combination of 35% hydrogen peroxide and 1 mM iron sulfate), to induce the oxidative stress of human saliva sample. Briefly, 90 µL of saliva was incubated with 10 µL of Fenton reagent (5 µL of 35% H_2O_2 + 5 µL of 1 mM iron sulfate) in the presence of 10 µL of either *Apis mellifera* propolis (AMP) extract or *Melipona favosa* propolis (MFP) extractor. After the incubation, the salivary TAA was assayed by the ABTS method (Re et al., 1999.

Total antioxidant activity

Total antioxidant activity was assayed by the Trolox equivalent antioxidant capacity (TEAC) (Re et al., 1999).

Total polyphenol content

Total polyphenol content in propolis was determined by the Folin-Ciocalteu colorimetric method (Singleton et al., 1999).

Total flavonoid content

Total flavonoid content in propolis sample was determined by the method of Woisky and Salatino (1998) with minor modifications

Statistical Analysis

The results are reported as the means ± SD. Statistical analysis of data was carried out by computer using SPSS 12.0 software. One-way ANOVA and Duncan post hoc multiple comparison tests were used to analyze data. P-values less than 0.05 were considered significant.

Results and Discussion

As is shown in table 1, saliva in the presence of dissolvent had 0.45 ± 0.09 mM TEAC. Fenton reagent decreased the salivary TEAC (0.22 ± 0.13 mM). However, when saliva sample was oxidized with Fenton reagent in the presence of (1/10) diluted AMP extract, there was an increase in salivary TEAC (1.14 ± 0.18 mM). MFP extract protected against oxidation and salivary TEAC was similar to that of saliva without oxidation.

Propolis extracts were reported to have antioxidant activity (Sheng et al., 2007; Moreira et al., 2008). To our best knowledge this is the first report that has shown that propolis can propect human saliva against Fenton reagent-induced oxidative stress. This observation could be explained by the fact that the ethanolic propolis extract used in this work had a relatively high content of polyphenols (184.81 ± 4.18 mg GAE/g of propolis) and flavonoids (91.27 ± 5.23 mg QE/g propolis) of AMP. These compounds are known as potent antioxidants, and the total phenols content has been considered the main responsible for the antioxidant activity of different propolis extracts (Kumazawa et al., 2004).

Table 1. Effect of AMP and MFP extract on the Trolox equivalent antioxidant capacity (TEAC) of human saliva treated by Fenton reagent

Samples	TEAC (mM)
Saliva + 95% ethanol	0.45 ± 0.09 (n = 6)
Saliva + Fenton reagent + 95% ethanol	0.22 ± 0.13 (n = 6)
Saliva + Fenton reagent + AM propolis extract	1.14 ± 0.18 (n = 6)
Saliva + Fenton reagent + MF propolis extract	0.43 ± 0.07 (n = 6)

Conclusions

In conclusion, AMP and MFP extracts could be used both to protect against oral oxidative stress and to improve the antioxidant status of oral environment.

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References

Al-Shaher, A., Wallace, J., Agarwal, S., Bretz, W., Baugh, D. 2004. Effect of propolis on human fibroblasts from the pulp and periodontal ligament. J Endod 30, 359-361.

Cheng, P. C., Wong, G. 1996. Honey bee propolis: prospects in medicine. Bee World 77, 8-15.

Gebara, E. C. E., Zardetto, C. G. D. C., Mayer, M. P. A. In vitro study of the antimicrobial activity of natural substances against S. mutans and S. sabrinus. Rev Odontol Univ São Paulo 10, 251-256.

Greabu, M., Battino, M., Totan, A., Mohora, M., Mitrea N., Totan, C., Spinu, T., Didilescu, A. 2007. Effect of gas phase and particulate phase of cigarette smoke on salivary antioxidants. What can be the role of vitamin C and pyridoxine? Pharmacological Reports 59, 613-618.

Ikeno, K., Ikeno, T., Miyazawa, C. 1991. Effects of propolis on dental caries in rats. Caries Research 25, 347-351.

Magro-Filho, O., Carvalho, A. C. 1990a. Application of propolis to dental sockets and skin wounds. Journal of Nihon Univ Sch Dent 32, 4-13.

Magro-Filho, O., Carvalho, A. C. 1990. Topical effect of propolis in the repair of sulcoplastics by the modified Kazanjian technique. Journal of Nihon Univ Sch Dent 36, 102-111.

Marcucci, M. C. 1995. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie 28, 71-76.

Martin, M. P., Pileggi, R. 2004. A quantitative analysis of propolis: a promising new storage media following avulsion. Dent Traumatol 20, 85-89.

Murray, M. C., Worthington, H. V., Blinkhorn, A. S. 1997. A study to investigate the effect of a propolis-containing mouthrinse on the inhibition of de novo plaque formation. J Clin Periodontal 24, 796-798.

Nagler, R. M., Reznick, A. Z. 2004. Cigarette smoke effects on salivary antioxidants and oral cancer novel concepts. IMAJ, 6, 691-694.

Park, Y. K., Koo, M. H., Abreu, J. A., Ikegaki, M., Cury, J. A. Rosalen, P. L. 1998. Antimicrobial activity of propolis on oral microorganisms. Current Microbiology 36, 24-28.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Bio. Med. 26, 1231-1237.

Sheng, J., Zhou, J., Wang, L., Xu, J., Hu, Q. 2007. Antioxidant activity of ethanol and petroleum ether extracts. Eur. Food Res Technol. 225, 249-253.

Silva, F. B., Almeida, J. M., Sousa, S. M. 2004. Natural medicaments in endodontics – a comparative study of the anti-inflammatory action. Braz Oral Res 18, 174-179.

Silva, E. B., Silva, F. B., Franco, S. L., Ramalho, L. T. O., Peruchi, C. M. S. 2000. Propolis effects on the lamina propria of the rats oral mucosal histologic study. ROBRAC 9, 4-8.

Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299, 152-178.

Walker, C.B. 1996. The acquisition of antibiotic resistance in the periodontal microflora. Periodontology 2000 10, 79-88.