Modulatory activity of brazilian red propolis on chemically induced dermal carcinogenesis

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ABSTRACT

PURPOSE: To evaluate modulatory effects of a hydroalcoholic extract of Brazilian red propolis (HERP) on dermal carcinogenesis using a murine model.

METHODS: The HERP was used at concentrations of 10, 50 and 100 mg/kg (PROP10, PROP50 and PROP100, respectively) to modulate dermal carcinogenesis induced by the application of 9,10-dimetil-1,2-benzatraceno (DMBA) on the backs of animals.

RESULTS: The chemical compounds identified in HERP included propyl gallate, catechin, epicatechin and formononetin. PROP100 treatment resulted in significantly decreased tumor multiplicity throughout the five weeks of tumor promotion (p<0.05), and this concentration also resulted in the highest frequency of verrucous tumors (p<0.05). All of the tumors that developed in DMBA-treated animals were regarded as squamous cell carcinomas and were either diagnosed as non-invasive verrucous carcinomas or invasive squamous cell carcinomas (SCCs). The average score for malignancy was significantly lower in the PROP100-treated group than the non-treated group (p<0.05), but there was no difference between the other groups (p>0.05).

CONCLUSION: The oral administration of hydroalcoholic extract of Brazilian red propolis at a dose of 100 mg/kg had a significant modulatory effect on the formation, differentiation and progression of chemically induced squamous cell carcinoma in a murine experimental model.

Key words: DMBA. Squamous Cell Cancer. Skin Cancer. Rodents. Propolis
Introduction

Cancer chemoprevention represents the prevention or delay of carcinogenesis due to the ingestion of dietary or pharmaceutical agents. Due to the side effects associated with the long-term use of synthetic compounds for the prevention of skin cancer, research has been directed towards identifying chemopreventive compounds from natural products.

Propolis is a natural resinous hive product that honeybees manufacture by mixing their own waxes and salivated secretions with resins collected from the cracks of the tree bark and leaf buds. Moreover, propolis collected from different regions of Brazil has been shown to display distinct colors and chemical compositions depending on the local flora at the site of collection. A new type of Brazilian propolis, which is popularly known as “red propolis”, has been described and characterized. The major chemical constituents of this variety, as identified by high-performance liquid chromatography from ethanolic extracts, include the flavonoids pinocembrin, formononetin and isoliquiritigenin. Studies have demonstrated that Brazilian red propolis displays cytotoxic effects on cell lines derived from human malignant tumors.

The chemopreventive effects of chemical compounds on skin cancer have been assessed using experimental models of chemically induced dermal carcinogenesis. DMBA (7,12-dimethylbenz(a)anthracene) is a site- and organ-specific carcinogen commonly employed to experimentally induce skin cancer, and the topical application of DMBA has been shown to induce dermal carcinogenesis in mice. DMBA is a polycyclic aromatic hydrocarbon that is metabolized into dihydrodiol epoxide, a compound able to bind to and damage DNA. Therefore, DMBA-induced skin carcinogenesis is regarded as an ideal tool to evaluate the chemopreventive efficacy of different anti-cancer agents in rodent models.

The purpose of this study was to assess the antitumor activity of different doses of a hydroalcoholic extract of Brazilian red propolis (HERP) on dermal carcinogenesis induced by DMBA in a murine model.

Methods

Ethical principles for experiments in animals were applied in this study. The experimental protocols and procedures were previously approved by the University Tiradentes Animal Care and Use Committee (CEUA nº 020913).
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UFLC analysis was used to determine the chemical profile of HERP, and a representative UFLC chromatogram of HERP is presented in Figure 1. Peak 1 was identified as propyl gallate, peak 2 was identified as catechin, peak 3 was identified as epicatechin, and peak 4 was identified as formononetin. The concentrations of propyl gallate and catechin in HERP were 5.46 mg.g⁻¹ and 17.69 mg.g⁻¹, respectively. Formononetin appeared to be a significant compound in this propolis extract, although neither this compound nor epicatechin could be quantified because they were co-eluted with other compounds. In addition, peak 5 (a major concentration) was unable to be identified.
The oral administration of HERP did not delay the appearance of tumors, as skin tumors developed during the third week of tumor promotion following the initial carcinogen application in all of the DMBA-treated groups, regardless of HERP treatment. However, the highest dose of HERP (100 mg/kg) significantly decreased the tumor multiplicity throughout the five weeks of tumor promotion (p<0.05) (Figure 2).

The administration of 100 mg/kg HERP significantly altered the clinical presentation of the tumors (p<0.05), as the emergence of verrucous lesions was increased in PROP100-treated animals compared with the ulcerative pattern observed in the other DMBA-treated groups (Figures 3 and 4).

All of the tumors that developed in the DMBA-treated animals were regarded as squamous cell carcinomas and were diagnosed either as non-invasive verrucous carcinomas (VCs) or invasive squamous cell carcinomas (SCCs). Moreover, these carcinomas were classified as grade 1, grade 2 or grade 3 tumors. Histologically, VCs appeared as a well-differentiated proliferation of squamous cells, consisting of the combination of high papillomatosis and hyperorthokeratinization with mild cytological or architectural anomalies. Moreover, the proliferative pattern seemed to push back rather than invade the underlying tissue. Grade 1 SCCs were characterized by the proliferation of well-differentiated squamous cells containing only slightly enlarged, hyperchromatic nuclei with abundant amounts of cytoplasm. Moreover, these tumors often produced large amounts of keratin and led to the formation of extracellular...
keratin pearls. Grade 2 SCCs were characterized by moderately differentiated squamous cells with variable nuclear atypia and limited keratinization. Grade 3 SCCs presented as poorly differentiated tumors with greatly enlarged, pleomorphic nuclei and a high degree of atypia and frequent mitoses. Moreover, keratin production in these cells was markedly reduced or absent (Figure 5).

No grade 4 SCCs (highly atypical sarcomatoid carcinomas) were detected in this study. The average scores for malignancy grading were significantly lower in PROP100-treated animals than TUM animals (p<0.05), but there were no differences between the other groups (p>0.05) (Figure 6).

The frequency of tumor involvement in notable dermal anatomical structures, such as the invasion of the perineural space, the dissociation of muscle fibers and the formation of tumor emboli within lymphatic vessels (Figure 5), was evaluated. As shown in Figure 7, no significant difference in either the frequency of muscle invasion and dissociation or the infiltration of peripheral nerve sheathes was observed between groups (p>0.05).

However, tumor emboli formation was significantly less frequent in the HERP-treated groups compared with the untreated groups, particularly at the dose of 100 mg/kg (p<0.01).

Discussion

According to Daugsch et al.4, formononetin is one of the major components present in Brazilian red propolis, and several studies have used formononetin as a chemical and/or biological marker. Yang et al.12 found that formononetin and its derivatives exhibited potent antiproliferative activities against two human tumor cell lines in vitro. Therefore, the identification of formononetin in our sample highlights the potential use of HERP as a chemoprotective agent.
The low number of DMBA-induced tumors observed in animals treated with the highest dose of HERP suggests that Brazilian red propolis had a chemopreventive effect against skin carcinogenesis. Furthermore, the number of verrucous tumors was also significantly increased in animals treated with 100 mg/kg HERP compared with the more ulcerative/infiltrative tumors that developed in the other treatment groups. These data suggest that Brazilian propolis may affect the growth pattern of tumors by stimulating the emergence of more exophytic (and less invasive) variants of SCC. Moreover, these morphological findings may influence the prognosis of cutaneous SCC, as it has been shown that exophytic malignant epithelial tumors display a lower tendency to generate lymph node metastases and therefore often lead to increased survival rates.

In the current study, significantly lower histological malignancy scores were observed for the tumors in HERP-treated animals, which supports the potential modulatory effect of Brazilian red propolis on the dynamics of tumor differentiation. These biological effects may be related to the chemical composition of the bee product, as it has been recently demonstrated that formononetin represents the major chemical constituent of Sergipe-derived Brazilian red propolis\(^4\), and previous reports have also stated that this isoflavonoid possesses potent antioxidant activity\(^12\). However, studies have demonstrated that following consumption by mammals, formononetin is metabolized into daidzein, an aglycon isoflavonoid with distinct antitumor activities against breast\(^15\) and ovarian cancer-derived cell lines\(^16\). These antitumor cytotoxic effects of daidzein are thought to be related to the inhibition of enzymes, such as DNA topoisomerase III, S6 ribosomal kinase, phosphoinositide 3-kinase and protein kinase C, which are proteins involved in the biochemical regulatory processes of cell proliferation and differentiation, and the generation of reactive oxygen species\(^17\).

In this study, the epithelial tumors developed in animals treated with 100 mg/kg HERP exhibited better differentiation and high keratinization rate values compared with the other groups, and these histological findings may also be associated with the biological effects of daidzein. It has been reported that daidzein induces mitochondrial disruption and promotes apoptosis in tumor breast cells in vitro (MCF-7) by inhibiting the bcl-2 gene, stimulating bax transcription, and promoting the release of cytochrome C into the cytosolic environment\(^18\). As keratinization is an apoptosis-related phenomenon that occurs in fully differentiated keratinocytes, it is possible that the development of more keratinized tumors in HERP-treated animals could be related to dietary propolis-derived daidzein. However, although we identified formononetin in our HERP samples, we were unable to assess how much of this compound was metabolized into daidzein. Thus, further investigations are necessary to clarify whether this metabolite is involved in the modulation of cell proliferation and the differentiation of experimental cutaneous SCC.

No significant difference was observed between groups regarding the frequency of muscular and perineural invasion. However, the frequency of tumor cell emboli formation was significantly decreased in HERP100-treated animals. Moreover, lymph node metastases resulting from lymphatic vascular dissemination of tumor cells are regarded as the most relevant histological feature for predicting the biological behavior of SCCs\(^19\). Although no previous studies have investigated the antimetastatic properties of red propolis, the results of the current study suggest that such an antimetastatic effect may be related to the type of tumor differentiation and the less invasive tumors observed in HERP-treated animals. In fact, the invasiveness of SCCs is closely related to the metastatic potential of the tumor\(^20\), but further studies are required to clarify the precise mechanisms underlying the red propolis-induced downregulation of vessel infiltration by squamous tumor cells.

In conclusion, oral administration of HERP at a dose of 100 mg/kg reduced the number of DMBA-induced skin SCCs, stimulated the formation of less invasive and more differentiated tumors, and reduced tumor emboli formation. These data suggest that Brazilian red propolis may exert an important modulatory effect on chemically induced dermal carcinogenesis.

**References**


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Acknowledgements

To Tiradentes University for the fellowship and the beekeepers, especially Jucilene Santana dos Santos, for the propolis sample.

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Received: Oct 15, 2013
Review: Dec 16, 2013
Accepted: Jan 20, 2014
Conflict of interest: none
Financial source: Foundation for Research and Technological Innovation Support from Sergipe (FAPITEC/SE)

Research performed at Laboratory of Morphology and Structural Biology (LMBE), ITP (Science and Technology Institute), Tiradentes University (UNIT), Aracaju-SE, Brazil. Part of Master degree thesis, Postgraduate Program in Health and Environment. Tutor: Prof. Dr. Ricardo Luiz Cavalcante de Albuquerque Júnior.