SHORT COMMUNICATION

Antiproliferative activity of aqueous and polyphenol-rich extracts of *Larrea divaricata* Cav. on a melanoma cell line

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**ABSTRACT**

Most of the deaths from skin cancer are caused by melanoma, a malignancy in which STAT3 plays a crucial role. The inhibition of STAT3 is considered a potential target to induce cell death, tumor regression and metastasis inhibition. The objective of this work was to evaluate the activity of the aqueous extract of *Larrea divaricata* (Aq), a fraction rich in polyphenols (EA), and the isolated compound quercetin-3-methyl ether (Q3ME) on B16F10 melanoma cells. The effects of Aq, EA and Q3ME were assessed on B16F10 cells by determining the proliferation, viability, apoptosis induction and the expression and phosphorylation of STAT3. The phytochemical composition of the extracts was determined by High Performance Liquid Chromatography. Aq, EA and Q3ME presented antiproliferative activity on B6F10 cells through p-STAT3 inhibition and early and late apoptosis induction (EC50 EA = 0.1 μg/ml; Aq = 316 ± 30 μg/ml; Q3ME = <0.1 μg/ml). *L. divaricata* could be considered for the development of adjuvant phytotherapies in melanoma treatment.

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1. Introduction

According to the World Health Organization (WHO), 80% skin cancer deaths are due to melanoma. In 50-90% of melanomas, the Janus kinase signal transducer and activator of transcription, STAT3, is constitutively activated and is considered a potential treatment target to induce cell death, tumor regression and inhibition of metastasis (Kortylewski et al. 2005; Kamran and Gude 2013).

Polyphenol-rich natural products have been reported to exert an antiproliferative activity on melanoma cells (Stevanato et al. 2014; Tong and Young 2014). Since the synthetic agents used for melanoma treatment can cause severe adverse effects, natural safe agents obtained from plant extracts should be considered. Larrea divaricata (Zygophyllaceae) is an autochthonous South American plant widely distributed in Argentina and used in popular medicine for the treatment of inflammatory diseases and cancer. This species produces polyphenol compounds such as hydroxycinnamic acid, flavonoids and lignans (Lorenzo et al. 2020). It has previously been demonstrated that an aqueous extract (Aq) of the leaves and a polyphenol-rich sub-fraction obtained by extraction with ethyl acetate (EA) exert antiproliferative effects on the EL-4 lymphoma cell line (Martino et al. 2013, 2016). However, the effect of these extracts has not been studied on melanoma cells. This work aimed at elucidating the anti-proliferative mechanism of action of Aq and EA on melanoma cells. The results obtained would be useful to develop natural novel phytomedicines to be used along with the adjuvant therapy for melanoma.

2. Results and discussion

In this work, an aqueous extract and a polyphenol-rich extract from L. divaricata exerted antiproliferative effects on a melanoma cell line by inhibiting the phosphorylation of STAT3, and inducing early and late apoptosis.

The lignan nor-dihydroguaiaretic acid (NDGA) (retention time: 47 min), 4-hydroxybenzoic acid (4-HBA) (retention time: 12 min), together with other flavonoids (retention times between 24-25 min) were identified and quantified by HPLC-UV in Aq and EA. Quercetin-3-methyl ether (Q3ME) was only detected in EA (chromatograms not shown). As it can be seen in Table S1, the amount of polyphenols, Q3ME, NDGA and the 4-HBA, were greater in EA than in Aq.

Aq and EA inhibited B16F10 cell proliferation in a concentration–response manner and decreased cell viability (Figures S1A and B), but a dependency of the latter effect with the extract concentration could be established neither with Aq nor with EA. The highest inhibitory capacity was observed with EA (Table S2). All compounds exerted antiproliferative activity \( p < 0.001 \)–\( p < 0.0001 \). 4-HBA was the least active compound and Q3ME, which also decreased cell viability \( p < 0.0001 \), was found to be the most potent (Figure S1C, D and E, Table S2). Taking into account these results, Q3ME was used for further studies.

The flow cytometry analysis demonstrated that Aq, EA and Q3ME at 10 \( \mu g/ml \) were able to induce a significant decrease of cell viability, an increase in the number of cells in early apoptosis \( p < 0.0001 \), late apoptosis \( p < 0.01 \) as well as necrotic ones \( p < 0.05, p < 0.001 \) (Figure S1E). It had been previously demonstrated that Aq inhibited the proliferation of the BW 5147 lymphoma cell line and that EA inhibits EL-4 lymphoma cell line by inducing apoptosis mediated by ROS and NO (Davicino et al. 2010; Martino et al. 2013,
Q3ME, 4-HBA and NDGA had also shown antiproliferative effects on dermal tumor cells and breast cancer cells (Kubow et al. 2000; Li et al. 2012; Wang et al. 2018).

It is known that the activation of STAT3 leads to the synthesis of molecules involved in malignant transformation and since several clinical trials are being undertaken to study the effectiveness of STAT3 inhibitors, including natural molecules (Siveen et al. 2014), the interaction of the most active drugs, EA and Q3ME, with STAT3 was analyzed.

The STAT3 proliferative dependency of the melanoma cell line was confirmed, since the STAT3 inhibitor 5,15 DPP decreased cell proliferation and STAT3 phosphorylation, while the pro-inflammatory cytokine IL-6 exerted opposite effects (Figure S2 A, C and D). The anti-proliferative effect of EA and Q3ME was mediated by the inhibition of STAT3 phosphorylation (Figure S2 C and D). The latter finding was confirmed by the fact that IL-6 was able to revert the antiproliferative effect induced by EA and Q3ME (Figure S2B). The inhibition of STAT3 phosphorylation could be related to the induction of apoptosis. It has previously been reported that quercetin regulates the STAT3 pathway in melanoma cells by inhibiting its phosphorylation/activation (Cao et al. 2014). Plant extracts containing quercetin and kaempferol exert antiproliferative effects on B16F10 melanoma cells by inducing late apoptosis and inhibiting the phosphorylation of STAT3. These effects were observed with the herbal formula “Huai-Hua-San”, which contains Sophorae flos and Gardeniae fructus ethanolic extracts, which were found to decrease viability with an EC50 value of around 400 μg/ml, a value that was higher than that found for L. divaricata extracts (Li et al. 2021).

3. Experimental
See supplementary material.

4. Conclusions
The results presented in this work serve as the basis for future in vivo studies in induced melanoma models to determine the effect of aqueous or polyphenol-rich extracts form L. divaricata as coadjutant phytotherapies for the treatment of this disease.

Competing interests
The authors declare no competing interests.

Disclosure statement
No potential conflict of interest was reported by the authors.

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