Hydroalcoholic leaves extract of Vaccinium ashei Reade promotes cell cycle arrest and apoptosis on T-cell acute lymphoblastic leukemia

Dalila Meneghetti, Verciane Schneider Cezarotto, Natália Paiva do Nascimento, Natacha Azussa Migita, Juliana Ronchi Corrêa, Maria Francesca Ricci, Lilian Girotto Zambaldi, José Andrés Yunes and Leonardo Luís Artico

Universidade Regional Integrada do Alto Uruguai e das Missões, Frederico Westphalen, RS, Brazil; Programa de Pós-graduação em Genética e Biologia Molecular, Universidade Estadual de Campinas, Campinas, SP, Brazil; Centro Infantil Boldrini, Campinas, SP, Brazil; Departamento de Genética Médica, Faculdade de Ciências Médicas, Universidade Estadual de Campinas, Campinas, SP, Brazil

ABSTRACT

Vaccinium ashei Reade, popularly known as Rabbiteye blueberry, has several therapeutic properties attributed to the phenolic compounds present in its leaves and fruits. Here, we sought to evaluate the effects of the hydroalcoholic extract from V. ashei leaves (Bluegem cultivar, VAB) in T-cell Acute lymphoblastic leukemia (T-ALL). The VAB extract was toxic to T-ALL cells at the ~60 μg/ml concentration. T-ALL cell death occurred through apoptosis. VAB extract was found to induce micronuclei formation, p53 pathway activation, and cell cycle arrest. Those mutagenic effects were evidenced through microscopy analysis and molecular p53 pathway activation. A series of phenolic compounds were identified in VAB extract by mass spectrometry, such as vanillic acid, catechin, caffeic acid, chlorogenic acid, rutin, coumaric acid, taxifolin, quercetin and naringenin, some of which are presumed to induce DNA damage. In conclusion, the V. ashei leaves extract may have important secondary metabolites with antileukemic properties.

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CONTACT

Leonardo Luís Artico lla.unicamp2017@gmail.com

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1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer (Katz et al. 2015). Many of the therapeutic drugs in use against ALL derive from leading compounds first isolated from plants, highlighting the importance of medicinal plants as a resource for new drug development (Singh et al. 2016; Bernardini et al. 2018). In this context, Vaccinium ashei Reade, also known as Rabbiteye blueberry, has drawn considerable attention in the phytochemistry field due to its high content of bioactive compounds (Sousa et al. 1995; Goldmeyer et al. 2014).

Although the commercial interest of this species is mainly focused on its fruit, studies carried out with V. ashei leaves extracts found high levels of phenolic compounds (Li et al. 2013), such as anthocyanins, procyanidins, acids and phenolic esters and flavonoids (Cezarotto et al. 2017). Several phenolic compounds have already been described for their in vitro and in vivo cytotoxic effects against leukemia (Lin et al. 2009; Bourogaa et al. 2011; Olivas-Aguirre et al. 2020). Therefore, in view of the growing interest for new compounds with potential anticancer effects, we investigated the cytotoxic properties of the hydroalcoholic extract of V. ashei leaves – Bluegem cultivar (VAB) and its mechanism of action in T-ALL cell lines.

2. Results and discussion

Even though V. ashei is known worldwide for its economic potential, the scientific nomenclature remains unclear according to The Plant List platform (http://www.thep plantlist.org). This inconsistency might be due to the broad variability of species, in terms of genetic (characterized by the divergence in the chromosomes number) and morphological aspects (Ballington 2006). In our study, we used leaf extract from V. ashei – Bluegem (VAB), a cultivar widely studied and genetically improved by Embrapa Clima Temperado in Brazil (Antunes et al. 2004; Antunes and Madail 2005; Antunes and Raseira 2006; Cezarotto et al. 2017).

Our data demonstrate that the hydroalcoholic VAB extract has cytotoxic activity against two T-ALL cell lines (Jurkat and Molt4) with half maximal inhibitory
concentration (IC$_{50}$) around 60µg/ml. The IC$_{50}$ for normal T-lymphocytes, previously treated with anti-CD3/CD28 beads in order to activate their proliferation, was approximately 2 times higher (122 µg/ml), demonstrating some degree of selectivity of this extract against leukemic cells (Figure S1). Knowing that many cytotoxic compounds act by deregulating cell mitosis (Singh et al. 2016), we performed cell cycle analysis to elucidate the mechanism by which extract from VAB leaves might exert its cytotoxic effect. The results obtained in Jurkat and Molt4 cells indicated that VAB extract induced cell cycle arrest at the G2/M phase, and an increase in the Sub-G0/G1 fraction suggestive of apoptosis (Figure S2A and S2B). In fact, apoptosis induction was confirmed by Annexin-V/7AAD staining (Figure S2C and S2D).

Some secondary metabolites of plants, such as flavonoids and phenolic compounds, have already been described as inducers of DNA damage, cell cycle arrest and apoptosis in diverse cancer cell models, including leukemia (Ghorbani et al. 2012; George et al. 2017; Olivas-Aguirre et al. 2020). Cezarotto et al. (2017) reported that the extract from V. ashei VAB leaves has high levels of phenolic compounds (mainly chlorogenic acid) and flavonoids (such as rutin). Here, by using mass spectrometry analysis (LC-ESI-MS/MS) we confirmed the presence of chlorogenic acid and rutin in the VAB leaf extract, in addition to other 7 phenolic compounds: vanillic acid, catechin, caffeic acid, coumaric acid, taxifolin, quercetin, and naringenin (Table S1).

A recent review describes how p53 pathway activation followed by apoptosis induction is one of the main mechanisms of actions of phenolic compounds against cancer cells (Fakhri et al. 2020). In line with this notion, treatment of ALL cells with the VAB leaf extract resulted in p21, p27 and p53 upregulation as some of the earliest events recorded (Figure S3A). In addition, we observed increased mRNA expression of the $P21$ and $PUMA$ genes, which are positive regulators of apoptosis induced by p53 (Yu and Zhang 2003), 24 h after VAB extract treatment (Figure S3B). These proteins are important inhibitors of cyclin/CDK complexes, especially in response to DNA damage events. Accordingly, VAB extract treatment resulted in decreased CDK4 and CDK6 expression at a later time point, and lower Retinoblastoma protein (Rb) phosphorylation (Figure S3A), which is a critical regulator of cell cycle progression at the G1 checkpoint. DNA damage is known to activate the p53 pathway, triggering cell-cycle arrest and apoptosis. Treatment of T-ALL cell lines with IC$_{50}$ concentrations of the VAB leaf extract was found to be highly mutagenic, as evidenced by gross chromosomal alterations and micronuclei formation. Surprisingly, proliferating normal T-lymphocytes were apparently not vulnerable to the VAB extract-induced mutagenicity (Table S2 and Figure S3C). The role of p53 and PUMA in inducing apoptosis due to mutagenic/genotoxic effects is well documented (Chipuk et al. 2005; Li et al. 2011; Ju et al. 2015), along with the potential for some phenolic compounds to activate this signaling cascade (Fakhri et al. 2020).

We believe that secondary metabolites present in the VAB extract are responsible for the apoptotic effects on leukemia cells in this study. Intriguingly, normal T lymphocytes seemed resistant to the deleterious effects of these compounds. These results must be taken in mind carefully as VAB extract has other phenolic compounds not fully studied which might also influence cancer pathways, thus, future research with individual compounds is warranted. Finally, this works contribute to increase the
scientific evidence on the use of phenolic compounds in cancer research and shows the importance of secondary metabolites that can affect these oncogenic pathways.

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**ORCID**

Natacha Azussa Migita [http://orcid.org/0000-0002-1326-3841](http://orcid.org/0000-0002-1326-3841)

Juliana Ronchi Corrêa [http://orcid.org/0000-0003-1502-0293](http://orcid.org/0000-0003-1502-0293)

José Andrés Yunes [http://orcid.org/0000-0002-1316-3525](http://orcid.org/0000-0002-1316-3525)

Leonardo Luís Artico [http://orcid.org/0000-0003-4691-2446](http://orcid.org/0000-0003-4691-2446)

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