Berries and leukemia: a systematic review of experimental studies and the \textit{in vitro} effect of ellagic acid on chronic myeloid leukemia cells

Frutas vermelhas e leucemia: uma revisão sistemática de estudos experimentais e o efeito \textit{in vitro} do ácido elágico sobre células de leucemia mieloide crônica

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ABSTRACT
Diet is directly involved in cancer etiology, influencing positively or negatively. The aim of this study was to conduct a systematic review on the effect of berries on leukemia, identifying the main bioactive compounds involved in biochemical and molecular mechanisms through which they act and also to test the \textit{in vitro} effect of ellagic acid, one of the phytochemicals found in berries, on leukemia cells. The work was subdivided into two parts: the first consisted of analysis of abstracts in Pubmed / Medline databases using MeSH (Medical Subject Headings) terms, synonyms, related and free terms, such as: leukemia, berries, anthocyanins, ellagic acid and flavonoids resulting in 274 articles. After abstract analysis, based on the eligibility criteria, this number was reduced to 21 articles. The second part was the \textit{in vitro} investigation of the effect of ellagic acid by the MTT reduction in chronic myeloid leukemia cell lines (CML) resistant or not to chemotherapeutic agents.

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vitro, in vivo and ex vivo studies associated the bioactive compounds present in berries with anticancer effect, acting mainly by induction of metabolic enzymes, modulation of gene expression and cell proliferation, chemotherapeutic resistance, free radical scavenging and induction of apoptosis. The present work also highlights the induction of collateral sensitivity by ellagic acid, since this compound was able to preferentially act on chemotherapeutic resistant cells. However, it is emphasized that animal models and clinical trials are for establishing the main mechanisms of action and possible dosages of berries intake daily or by dietary supplementation that could contribute to leukemia's treatment.

**Key word:** leukemia, berries, anthocyanins, ellagic acid and flavonoids.

**RESUMO**

A dieta está diretamente envolvida na etiologia do câncer, influenciando positivamente ou negativamente. O objetivo deste estudo foi realizar uma revisão sistemática sobre o efeito das frutas vermelhas na leucemia, identificando os principais compostos bioativos envolvidos nos mecanismos bioquímicos e moleculares por meio dos quais atuam e também testar o efeito in vitro do ácido elágico, um dos fitoquímicos encontrados em frutas vermelhas, sobre células de leucemia. O trabalho se subdividiu em duas partes: a primeira consistiu em análises de resumos nas bases de dados Pubmed / Medline utilizando-se termos MeSH (Medical Subject Headings), sinônimos, termos relacionados e livres, tais como: leucemia, frutas vermelhas, antocianinas, ácido elágico e flavonoides, resultando em 274 artigos. Após análise dos resumos, com base nos critérios de elegibilidade, esse número foi reduzido para 21 artigos. A segunda parte tratou-se da investigação in vitro do efeito do ácido elágico por meio do ensaio de redução de MTT em linhagens celulares de leucemia mieloide crônica (LMC) resistentes ou não a quimioterápicos. Estudos in vitro, in vivo e ex vivo associaram os compostos bioativos presentes nas frutas vermelhas com efeito anticâncer, atuando principalmente pela indução de enzimas metabólicas, modulação da expressão gênica e proliferação celulares, resistência quimioterápica, eliminação de radicais livres e indução de apoptose. O presente trabalho ressalta, ainda a indução de sensibilidade colateral pelo ácido elágico, uma vez que esse composto foi capaz de atuar preferencialmente sobre células resistentes a quimioterápicos. No entanto, destaca-se que modelos animais e ensaios clínicos são cruciais para o estabelecimento dos principais mecanismos de ação e possíveis dosagens diárias de consumo de frutas vermelhas ou suplementação dietética que podem contribuir para o tratamento da leucemia.

**Palavras chave:** leucemia, frutas vermelhas, antocianinas, ácido elágico e flavonoides.

**1 BACKGROUND**

Cancer can be defined as a non-communicable disease characterized by uncontrolled cell proliferation. According to the International Agency for Research on Cancer/ World Health Organization (IARC/WHO), cancer is a major cause of morbidity and mortality worldwide, with approximately 14 million new cases and 8.2 million deaths related to this disease and an expectation of 70% increase in new cancer cases in the coming decades (STRATTON, 2011). Its occurrence depends on multiple factors, among which dietary habits and lifestyle factors play an important role. Its development results from alterations
of cellular DNA which accumulate over time due to the ability of these damaged structures to escape from cellular control mechanisms, such as cell cycle arrest and inhibition of proliferation (FERLAY et al., 2012).

Leukemia is a clonal disorder that occurs in a heterogeneous group of haematopoietic progenitor cells which loses their abilities to normally differentiate and respond to normal cellular regulators (ESTEY & DOHNER, 2006). Understanding of chemoresistance mechanisms is an important step in overcoming obstacles to a successful therapeutic intervention. Many drugs used in cancer therapy have been developed from plants, including plant foods, due to its rich composition on bioactive compounds. Among common foods, berries are known for their nutrients and phytochemicals profile, associated with the reduced risk for various diseases (NILE & PARK, 2014). Berries include blackberries, blueberries, cranberries, strawberries, raspberries, and others such as jabuticabs (*Plinia cauliflora*).

The benefits of berries can be due to the combined action of micronutrients with the variety of polyphenols already identified (ZHAO, 2007). Polyphenols comprise different classes of compounds, such as those derived from hydroxybenzoic and hydroxycinnamic acids, anthocyanins, proanthocyanins, flavonoids, flavonones, flavonols, flavonones, stilbenes and lignans, all present in different concentrations, in berries. Anthocyanins represent one of major phytochemicals present in most colorful berries and have been suggested as potential chemoprotective agents and a large and growing body of evidence from *in vitro* cell culture studies and *in vivo* animal model tumor systems (STONER & SEERAM, 2011). Many of these bioactive compounds have shown some protective effect against cancer by induction of metabolic enzymes, gene modulation, chemotherapeutic resistance control, induction of apoptosis, among others, even in leukemia models (SEERAM, 2008). Therefore, the aim of this study is to identify the possible role of berries in chemoprevention and chemoprotection against leukemia through a systematic review of *in vitro*, *in vivo* and *ex vivo* studies and also to test the *in vitro* effect of ellagic acid, one of the phytochemicals found in berries, on leukemia cells.

## 2 METHODS

**PART 1: SYSTEMATIC REVIEW**
2.1.1 Search strategy and Study Selection

Searches were performed on the electronic databases MEDLINE via PUBMED. A hand-searched was also done in the reference list of relevant publications and those which met the inclusion criteria were included. The initial search date was October 2015 with an update in April 2020, using the search strategy initially defined.

The following keywords, “leukemia” and “berries”, and all descriptors were identified by searching the Medical Subject Headings Terms - MeSH Terms - (MeSH). The established terms, synonyms, related and free terms of the search strategy are represented in (Table, Supplementary material), and were defined based on the elements of the PICO strategy where P is by definition population and the descriptions are leukemia cells (in vitro and ex vivo studies), I is by definition interventions and the descriptions are bioactive compounds present in berries, C is by definition control and doesn’t have description and O is by definition outcome and the descriptions are the effects of berries on leukemia. There was no restriction of language or date of publication applied to studies.

To guarantee the quality of the search, the included studies met the following criteria: (1) not being epidemiological and review papers; (2) had used as intervention berries fruits or individual compounds isolated from berries; (3) had been conducted on any kind of leukemia; (4) for the duplicate articles, the newest or most informative one was selected, and (5) articles that did not have enough information in the titles and abstracts were excluded. The search and inclusion criteria analysis were made separately by two authors (Juliana Garcia Borges Fernandes and Ana Luisa Kremer Faller) with no disagreement between them.

2.1.2 Data Extraction

Independently, each author listed the following information from the articles selected: first author’s name, publication year, study characteristics, cell type, compounds, doses used and results obtained. Any disagreements were resolved by discussion and the third and fourth researchers (Julia Quarti and Eliane Fialho) were consulted.

PART 2: EXPERIMENTAL

2.1.3 Reagents
All reagents used in this study were of analytical grade. RPMI-1640, β-mercaptoethanol, ellagic Acid, vincristine (VCR), daunorubicin hydrochloride (DNR), dimethylsulfoxide (DMSO), and 3,4,5-dimethiazol-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA); penicillin and streptomycin were from Invitrogen (Carlsbad, CA, USA); fetal bovine serum from Cultilab (Campinas, SP, Brazil).

2.1.4 LMC Cell Lines

In this study we used three leukemia cell lines: a chemotherapeutic-sensitive parental cell line, K562 and 2 two MDR cell lines, Lucena-1 and FEPS, that were derived from K562. K562 cells were originated from a patient with blast crisis of chronic myeloid leukemia and are characterized by expressing the chimeric oncogene BCR-ABL on the Philadelphia chromosome (KOEFFLER & GOLDE, 1980).

Lucena-1 and FEPS cells were developed in the Laboratório de Imunologia Tumoral of the Universidade Federal do Rio de Janeiro and were developed by continuous exposure of K562 cells to increasing concentrations of cytotoxic drugs, vincristine sulfate (VCR) for Lucena-1 and daunorubicin hydrochloride (DNR) for FEPS (RUMJANEK et al., 2001; DAFLON-YUNES et al., 2013). Lucena-1 cells present five times more copies of the MDR1 gene (encoding P-gp) than its K562 parent cell line (RUMJANEK et al., 2001). FEPS cells have higher P-gp expression and activity than Lucena-1 and also expresses the multidrug resistance-associated protein (MRP-1) (DAFLON-YUNES et al., 2013).

2.1.5 Cell Cultures

K562, Lucena-1 and FEPS cells were maintained in RPMI-1640 medium, pH 7.4, supplemented with 50 mmol/L β-mercaptoethanol, 25 mmol/L HEPES, 60 mg/L penicillin, 100 mg/L streptomycin and 10% fetal bovine serum. Lucena-1 and FEPS were maintained with 60 nM of VCR and 500 nM of DNR, respectively. To perform the experiments, the drugs were removed for three days from the cells culture. All cells were passaged at a concentration of 2 × 10^4 cells/mL every three or four days and kept at 37 °C in a 5% CO₂ humidified environment.

2.1.6 Cytotoxicity assay - MTT
K562, Lucena-1 and FEPS (2 × 10^4 cells/ mL) were incubated for 72 and 96 hours with ellagic acid (5–25 µM) diluted in 0.25% dimethyl sulfoxide (DMSO). Cell viability was assessed by 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) colorimetric assay. For this, 20 µL of MTT, diluted in Phosphate-Buffered Saline (PBS), were added at a final concentration of 0.5 mg/ mL in each well of a 96-well microplate. The plates were then kept at 37°C, 5% CO₂ for 3 hours. After centrifugation, 200 µL of DMSO were added into the wells in order to dissolve the dark blue crystals formed by the reduction of MTT. The absorbance of the converted dye in living cells was measured at a wavelength of 492 nm. The experiments were performed in triplicate. The IC₅₀ values were calculated from dose response curves; the IC₅₀ was defined as the concentration of drugs that reduced the number of viable cells to 50% of the control. GraphPad Software 5.0 was used for the IC₅₀ calculations.

### 2.1.7 Statistical Analysis

The results of the experiments were submitted to descriptive statistical analysis, mean and standard error, analysis of variance (ANOVA) followed by Tukey's post-test, using the program GraphPad Prism 5. Results with p values <0.05 were considered statistically significant.

### 3 RESULTS

#### PART 1: SYSTEMATIC REVIEW

#### 3.1.1 Literature Search

Two hundred and sixty-three potentially eligible studies were identified initially (263 records in the Pubmed database and 11 by hand-search selection). Of those, most articles (253 records) were excluded after titles and/or abstracts analyses. The reasons for exclusion were studies that used fruits other than berries (230 articles), and that addressed other types of cancer (23 articles). The detailed steps of study selection were shown in

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Figure 1.
3.1.2 Study Characteristics

The selected studies were published between 2002 and 2018 and were conducted in United States (5), Japan (4), China (2), France (2), Poland (2), Italy (1), Malaysia (1), Republic of Korea (1), Spain (1), The Netherlands (1) and United Kingdom (1). Of the 1 articles selected for this systematic review, 20 were \textit{in vitro} studies and 1 an \textit{ex vivo} study. Among the \textit{in vitro} studies, a wide diversity of cells was used, and both myeloid and lymphoid leukemias which are presented in Table 1. Studies included had the use of berries extracts as well as isolated phenolic compounds, such as ellagic acid, resveratrol, quercetin and anthocyanins. The main mechanisms identified were induction of apoptosis and autophagy, modulation of cellular proliferation, antioxidant action and chemoresistance control (Table 1).
Table 1: Systematic Review Articles

| AUTHORS                  | STUDY TYPE          | TREATMENT                                                                 | RESULTS                                                                                                                                 |
|--------------------------|---------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| HAGIWARA et al., 2010    | *In vitro* study with Ellagic Acid (EA) and retinoic acid (ATRA) in HL60 acute myeloid leukemia cells. | HL60 cells were treated with 5 to 25 µM of EA for 24 or 96 hours. Besides this, HL60 cells were exposed to 15 - 25 µM of EA in the presence of 0.2 µM or 2 µM of ATRA. | EA inhibited cell growth, induced cell cycle arrest in S-phase and apoptosis associated with caspase 3 activation in HL60 cells in a dose dependent manner. EA also potentiates ATRA induced differentiation in HL60 cells. |
| DAHLAWI et al., 2013     | *In vitro* study with Pomegranate Juice Extracts (PGJE) in lymphocytic leukemia (CCRF - CEM and MOLT3) and myeloid leukemia (HL60 and THP1) cell lines. | CCRF - CEM, MOLT3, HL60, and THP1 cell lines were treated with 6.25%, 12.5% and 25% of PGJE for 24, 48 or 72 hours. | Induction of caspase 3 dependent apoptosis by PGJE in a time and dose dependent manner for all leukemia cell lines. Beside this PGJE promoted S phase arrest at all concentrations. |
| SHARIF et al., 2010      | *In vitro* study with Red Wine Polyphenolic extract (RWPs) in Jurkat cells. | Jurkat cells were treated with 10 - 100 μg/ mL of RWPs for 24 hours. | RWPs inhibited the growth and proliferation of Jurkat cells and induced G0/G1 cell cycle arrest in a concentration-dependent manner. Moreover, RWPs induced caspase 3 dependent apoptosis and promoted ROS accumulation. |
| GE et al., 2013          | *In vitro* study with resveratrol in T-cell acute lymphoblastic leukemia cells (T-ALL, CEM-C7-14, MOLT4, JURKAT and CEM-C7-15). | CEM-C7-14, MOLT4, JURKAT, and CEM-C7-15 cell lines were treated with 200 µM of resveratrol for 24 and 48 hours. | Resveratrol inhibited the growth and induced caspase 3 dependent apoptosis in T-ALL cells in a time and dose dependent manner. It also induced cell cycle arrest in G0/G1 phase by increasing regulation of cyclin-dependent kinase (CDK) inhibitors p21 and p27 and down regulating cyclin A and cyclin D1. Besides this, resveratrol |
| Authors          | Study Details                                                                 | Findings                                                                 |
|------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------|
| WANG et al., 2005 | *In vitro* study with Lingonberries anthocyanins in human leukemia HL60 cells. | Human leukemia HL-60 cells were treated with indicated doses of lingonberry (control, 1:160, 1:180, 1:40 and 1:20) extracts for 18 hours. At dilution of 1:20 of extract of lingonberries was able to induce apoptosis (78% apoptotic cells). |
| KATSUBE et al., 2003 | *In vitro* study with berry extracts (low bush blueberry, high bush blueberry, cranberry, raspberry, strawberry, black currant, red currant, blackberry, bilberry and cowberry) and specific anthocyanidins from bilberry (pelargonidin, cyanidin, peonidin, delphinidin and malvidin) in HL60 human promyelocytic leukemia cells. | HL60 cells were treated with 2, 4 or 6 mg/mL of berry extracts for 24 or 48 hours. This study also determined the growth inhibitory and apoptosis inducing effects of the anthocyanidins (pelargonidin, cyanidin, peonidin, delphinidin and malvidin) at 50, 100 and 200 µM. Among tested extracts, bilberry was the most effective against HL60 cells by inducing apoptosis. Bilberry extract showed the largest amounts of anthocyanins, wherein 200 µM delphinidin, malvidin or cyanidin were the most capable of inhibiting HL60 cell growth and inducing apoptosis. |
| SZYMANOWSKA et al., 2018 | *In vitro* study with raspberry fractions and raspberry juice in J45.01 and HL60 cells. | J45.01 and HL60 cells were treated with crude extracts, anthocyanin-rich fractions and phenolic fractions from raspberry and raspberry juice for 24 hours. All examined extracts inhibited the viability of J45 cells more effectively than HL60, but the raspberry crude extract showed the greatest cytotoxic effect. |
| Author(s)          | Year       | Study Description                                                                 | Cell Lines                                                                                   | Treatment Details                                                                                                                      |
|-------------------|------------|------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------|
| FENG et al., 2007 |            | In vitro study with cyanidin-3-rutinoside (C-3-R) extracted and purified from the black raspberry (cultivar Jewel) in human leukemia and lymphoma cell lines HL60 (myeloblastic), MOLT-4 (lymphoblastic), Daudi (lymphoblastic) and CCRF-CEM (lymphoblastic). | HL60, MOLT-4, Daudi and CCRF-CEM were treated with 10-160 µM of C-3-R for 8, 18 and 32 hours. | C-3-R induced caspase-dependent apoptosis in HL60 cells in a time and dose dependent manner. C-3-R also induced apoptosis in other cell lines including MOLT-4, Daudi and CCRF-CEM, but had little toxicity in normal human cells by the MTT assay. Besides this, C-3-R promoted the activation of p38 MAPK and JNK, leading to the ROS accumulation and increasing oxidative stress in HL60 cells. |
| ASOU et al., 2002 |            | In vitro study with resveratrol in myeloid leukemia cell lines (HL60, NB4, U937, THP-1, ML-1 and Kasumi-1) and fresh samples from 17 patients with acute myeloid leukemia. | HL60, NB4, U937, THP-1, ML-1, Kasumi-1 and fresh leukemia cells were treated with 10 - 25 µM of resveratrol for 96 hours. U937 cells were also treated with the combination of 1,25(OH)_{2}D_{3} (0.1 - 10 nM) and resveratrol (25 µM) or either agent alone for 96 hours. NB4 cells were also treated with the combination of all-trans-retinoic acid (10 or 50 nM) and resveratrol (10 µM) or either agent alone for 96 hours. | Resveratrol inhibited the growth and induced death in all cell lines after exposure of 20 µM for 96 hours. In addition, 25 µM of resveratrol for 96 hours promoted typical monocytic differentiation. Besides this, the combination of resveratrol + 1,25(OH)_{2}D_{3} as well as resveratrol + all-trans-retinoic acid had an additive effect on the differentiation of U937 cells and NB4 cells, respectively. Resveratrol (20 µM for 96 hours) also induced differentiation of 8 samples of fresh leukemia cells. |
| FIMOGNARI et al., 2004 |            | In vitro study with cyanidin-3-o- | Jurkat cells treated with 12.5- | In Jurkat cells, 12.5 µg/mL of Cy-g was sufficient to |
| Glucopyranoside (Cy-g) in Jurkat and HL60 cell lines. | 200 µg/mL of Cy-g for 24 hours and HL-60 cells treated with Cy-g at the indicated doses for 8 and 30 hours. | Increase the number of cells displaying features of apoptosis at 200 µg/mL. Cy-g-induced apoptosis is associated with significant changes in p53 and bax proteins. When HL-60 cells were exposed to Cy-g for 8 h, recorded a dose-dependent increase in the fraction of apoptotic cells, at 200 mg/mL: 18% versus 8% in controls. It is noteworthy that HL-60 cells are p53 null. A link might exist between the ability of Cy-g to protect against reactive oxygen species damage and the pro-apoptotic effects observed in this study. |

**MERTENS-TALCOTT et al., 2003**

*In vitro* study with ellagic acid and quercetin as single compounds and in combination in human leukemia cells (MOLT4).

MOLT4 cell lines were treated with quercetin (5 or 10 µmol/L), ellagic acid (5 or 10 µmol/L) or quercetin + ellagic acid (5 or 10 µmol/L each) for 12, 24 or 48 hours.

Ellagic acid and quercetin achieve synergistic effects in the reduction of proliferation and viability in MOLT-4 cells. It also promoted cell cycle arrest in G0/G1 and S phases. Besides this, the polyphenols combination induced apoptosis by activation of caspase 3, which was confirmed by an isobolographic analysis for proliferation-reducing effects.

**MERTENS-TALCOTT et al., 2005**

*In vitro* study with ellagic acid, quercetin, and resveratrol as single compounds and in combination in human leukemia cells (MOLT4).

MOLT4 cell lines were treated with 10 µM of ellagic acid, quercetin or resveratrol as single compounds and in combination (10 µM each).

The results showed an additive effect for resveratrol and quercetin combination and for the combination of the three polyphenols, but the combination of ellagic acid with resveratrol exhibited a more than additive interaction. All of the
| Authors                        | Study Type                     | Treatment                                                                 | Findings                                                                                                                                                                                                                                                                 |
|-------------------------------|--------------------------------|---------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SAEDI et al., 2015            | *In vitro* study with Berberis vulgaris Fruit Crude Extract (BVFCE) in murine myelomonocytic leukemia cell line, WEHI-3. | Ellagic acid + resveratrol; Quercetin + resveratrol; Ellagic acid + Quercetin + Resveratrol for 10, 12, 24 or 48 hours. | Double combinations of polyphenols, resulted in a cell cycle arrest in the G0/G1 phase at 12 hours and in an S phase arrest after 24 hours, while the combination of all three polyphenols caused an arrest in the G0/G1 phase after 24 hours, but a synergistic effect of the polyphenols combinations was not observed. Caspase 3 activity assay indicates synergy for either of the combinations (ellagic acid + resveratrol and quercetin + resveratrol), but the combination of quercetin + resveratrol exceeded that of ellagic acid + resveratrol. |
| WANG et al., 2012             | *In vitro* study with Grape Seed Proanthocyanins (GSPs) in K562 leukemia cells. | K562 cells were exposed to GSP solution at 3.125 to 100 µg/mL for 72 hours. | At concentrations of 30 µg/mL, GSPs inhibited K562 cells proliferation and decreased intracellular ROS levels. After treatment with 50 µg/mL, a typical morphological differentiation was observed and cell cycle arrest in G1 phase at concentrations of 10 µg/mL and 30 µg/mL. |
| GARCIA-ALONSO et al., 2007    | *In vitro* study with phenolic-rich juice made from grapes, cherries and berries in human U937 cells were treated with phenolic-rich juice (10 - 200 µM) for 3 hours. | Pre-incubation of U937 cells with extracts of the phenolic-rich juice partially prevented cell death, abolished the DNA cleavage |
| Authors, Year          | Study Type | Cells/Tissue/Compliance | Results                                                                 |
|-----------------------|------------|-------------------------|-------------------------------------------------------------------------|
| Wang et al., 2007     | *In vitro* | Deerberry fruit extracts in HL60 cells | Decreased ROS generation. In a dose-dependent manner, deerberry fruit extracts decreased intracellular ROS levels, inhibited cells proliferation and induced apoptosis in HL60 cells. |
| Katsuzaki et al., 2002| *In vitro* | Anthocyanins isolated from skin of red grape | Anthocyanins inhibited cell growth in a dose dependent manner. At 1 mM concentration anthocyanins also induced apoptosis and ROS may play an important role in this type of cell death. |
| Sharif et al., 2012   | *In vitro* | Polyphenol-rich juice from black chokeberry | AMJ induces in Jurkat cells early and late apoptosis by generating a pro-oxidant signal and triggering mitochondrial membrane potential loss with a subsequent release of cytochrome c. AMJ treatment also induced apoptosis of different human lymphoblastic leukemia cells (HSB-2, Molt-4 and CCRF-CEM), but not in normal lymphocytes. |
| Kweon et al., 2010    | *In vitro* | Resveratrol in doxorubicin-resistant acute myeloid leukemia cells | Resveratrol induced cell death in AML2/DX300 cells in a dose and time-dependent manner. Treatment also downregulated the expression of the MRP1 gene and MRP1 protein activity in AML2/DX300 in a dose dependent manner. |
| Skupien et al., 2006  | *In vitro* | Blueberry extract | Blueberry extract was the most efficient against HL60. |
(blueberry, raspberries and strawberries) in HL60 promyelocytic cell line and its multidrug resistance sublines (HL60/VINC and HL60/DOX).

HL60/DOX cell lines were treated with three berries extracts at concentrations ranging from 0.1 to 2 g/L for 72 hours. Cells, but presented much lower activity towards resistant cells. In contrast, raspberry and strawberry extract exhibited high cytotoxic activity against both sensitive (HL60) and resistant (HL60/VINC and HL60/DOX) cell types.

WILMS et al., 2007

Ex vivo study with 168 healthy volunteers (114 females and 54 males) that consumed a blueberry juice with apple.

The volunteers consumed, for 4 weeks, 1 L of blueberry and apple juice (97 mg quercetin and 16 mg ascorbic acid)/day. Before and after intervention, quiescent peripheral blood lymphocytes were exposed ex vivo to oxidative stress and to the food carcinogen benzo[α]pyrene (B[α]P) for 1 and 18 hours, respectively. The intervention increased plasma antioxidant capacity and protection against oxidative DNA damage.

4 MAIN RESULTS

Cellular apoptosis is an active process of cell death that is important for tumor destruction. Caspases enzymes play an important role in the execution phase. The so-called initiators caspases such as caspases 8 and 9 directly or indirectly activate caspases 3 and 7, responsible for cleaving substrates, including Poly (ADP-ribose) polymerase (PARP), resulting in morphological markers of apoptosis changes (NUNEZ et al., 1998).

Myeloid leukemia cells (HL60) incubated with 2 μM of retinoic acid in the presence of ellagic acid (25 μM) for 96 hours inhibited cell growth, induced apoptosis by activation of caspases, and generated a slight accumulation of cells in S phase and reduction in G1 phase (HAGIWARA et al., 2010). Acetonitrile fraction of pomegranate juice extracts at concentrations of 6.25%, 12.5% and 25% induced apoptosis in a time and dose dependent
manner in four leukemia cell lines (CCRF-CEM, MOLT3, HL60 and THP1) following 24, 48 and 72 hours incubation by induction of caspase 3 activity (DAHLAWI et al., 2013). Similar results with activation of caspase 3 were observed when Jurkat cells were treated with phenolic rich extract (containing catechins, anthocyanins and phenolic acids) obtained from red wine at 100 μg/ mL for 24 hours (SHARIF et al., 2010).

Increasing evidences have demonstrated that apoptosis and autophagy may be triggered by common upstream signals and thus affect cancer development and therapy (YANG et al., 2011; WHITE & DIPAOLA, 2009). Autophagy as a type of cellular catabolic degradation response to nutrient starvation or metabolic stress, is considered as a survival mechanism induced in adverse conditions to maintain cell integrity, and it is also involved in a particular mode of cell death called autophagic cell death (MAIURI et al., 2007).

Only one study was found on apoptosis and autophagy. Resveratrol inhibited, in a dose and time-dependent manner, the growth of four T-cell acute lymphoblastic leukemia cell lines (T-ALL cells): the glucocorticoid sensitive (CEM-C7-14) and the glucocorticoid resistant (MOLT4, JURKAT, CEM-C7-15) (GE et al., 2013). In addition, 200 μM of resveratrol for 48 hours induced apoptosis by decreasing the expression of anti-apoptotic proteins and stimulating pro-apoptotic proteins expression, as well as inducing caspase 3 cleavage, also in a time-dependent manner. Autophagic vacuoles of T-ALL cells were observed under electronic microscopy after resveratrol treatment (GE et al., 2013).

Wang et al. 2005 found that lingonberry extract induced the apoptosis of human HL-60 leukemia cells in a dose-dependent manner, as indicated by morphology characteristics of apoptosis. Human leukemia HL-60 cells were treated with doses (control, 1:160, 1:180, 1:40 e 1:20) of lingonberry extracts for 18 h. At 1:20 extract concentration was able to induce 78% of cells in apoptosis. This effect may partially be due to its antioxidant properties by perturbing the favorable redox condition in cancer cells and its may be highly effective as a chemopreventive agent that acts by targeting specific oncogenes, such as AP-1 and NF-κB, suppressing cell neoplastic transformation and inducing cancer cell apoptosis.

When evaluating the effect of ten different berries extracts in HL60 leukemia cells, DNA fragmentation was observed, a typical process that indicates apoptosis (KATSUBE et al., 2003). Bilberry extract at concentrations of 4 mg/ mL and 6 mg/ mL incubated for 6 hours resulted in the highest decrease of HL60 cells viability, 84% and 88%, respectively. The group also showed that the glycosidic forms of delphinidine and malvinidine, two of the anthocyanins present, were responsible for effects observed in the cell line (KATSUBE
et al., 2003). Crude extract, anthocyanin-rich fractions and phenolic fractions from raspberry and raspberry juice were able to decrease viability of H60 and J45.01 cells, derived from human caucasian promyelocytic leukemia and human acute T cell leukemia, respectively. The J45.01 cell line was more sensitive, the value of EC$_{50}$ was significantly lower (0.35 mg FW/mL) than that noted for the H60 cell line (0.80 mg FW/mL) (SZYMANOWSKA et al., 2018).

With isolated compounds, Feng et al. (2007) demonstrated that 50 μM of cyanidin-3-rutinoside induced apoptosis in approximately 50% of HL60 cells within 18 hours of incubation and almost all cells were apoptotic at concentration of 120 μM. Cyanidin-3-rutinoside also induced apoptosis in other human leukemia/lymphoma cell lines, such as, MOLT4, Daudi and CCRF-CEM cells, but showed little toxicity against normal human cells (FENG et al. 2007). In another study, 20 μM of resveratrol inhibited proliferation and induced cell death in six myeloid leukemia cell lines (HL60, NB4, U937, THP-1, ML-1 and Kasumi-1) after exposure for 96 hours. In the same work, 19 bone marrow samples from acute myeloid leukemia patients were obtained from survivors of the Hiroshima atomic bomb. Each sample contained more than 95% leukemic cells, and eight of the nineteen samples reduced NBT (the reduction of NBT within them seems to be correlated with their phagocytic activity) after exposure to the same conditions described above, demonstrating the induction of differentiation of these cells by resveratrol (ASOU et al., 2002). Differentiation therapy could be used on leukemia's treatment, since cancer cells can be forced to differentiate and arrest proliferation, thereby controlling their malignant potential (GUTTERIDGE & HALLIWELL, 2000).

One another study with cyanidin-3-0-glycopiranoside (Cy-g) treatment to induce apoptosis in Jurkat and HL-60 cells was conducted. In Jurkat cells, even the lowest concentration tested (12.5 mg/mL) was sufficient to increase the number of cells displaying features of apoptosis at 200 mg/mL. When HL-60 cells were exposed to Cy-g for 8h, a dose-dependent increase in the fraction of apoptotic cells were observed. HL-60 cells were, therefore, more resistant to Cy-g-induced apoptosis than Jurkat cells (FIMOGNARI et al., 2004). In order to delineate the events leading to apoptosis elicited by Cy-g, the expression of different proteins were analyzed. Jurkat cells were treated for 24 hours with Cy-g 200 mg/mL and showed a marked increase in p53 and bax protein levels, whereas bcl-2 and c-myc levels were substantially unchanged. The analysis of protein levels in Jurkat cells showed that Cy-g-induced apoptosis is associated with significant changes in p53 and bax proteins,
suggesting that alterations in the levels of these proteins are directly responsible for the death signal delivered by Cy-g. It is noteworthy that HL-60 cells are p53 null. Thus, the induction of apoptosis in this system indicates that Cyg may exert its effects independently of the p53 gene. Cy-g is well known for its antioxidant properties, which have been demonstrated in several cell systems. A link might exist between the ability of Cy-g to protect against reactive oxygen species damage and the pro-apoptotic effects observed in their study (FIMOGNARI et al., 2004).

Isolated compounds and their combinations were used to determine whether these compounds may act in an additive, synergistic or antagonistic manner when inducing apoptosis (MERTENS-TALCOTT et al., 2003). To test this hypothesis, MOLT4 cells were incubated with ellagic acid (10 μM), quercetin (10 μM) and the combination of both at the same concentrations for 12, 24 and 48 hours. The association of the two compounds was more potent than the sum of individual effects, suggesting a synergistic mechanism promoting proliferation and viability reduction as well as induction of apoptosis (MERTENS-TALCOTT et al., 2003). Similar result was obtained by the same research group when testing ellagic acid (EA) at 68.4 μmol/ L, quercetin (Q) at 12.8 μmol/ L and resveratrol (R) at 54 μmol/ L individually for 24 hours or in combination (EA + Q + R) at a fixed concentration of 10 μmol/ L each for 10 hours (MERTENS-TALCOTT et al., 2005).

Another possible chemoprotective mechanism promote by berries is cell cycle arrest, thereby modulating cell proliferation. Saedi et al. (2015), demonstrated that the incubation of Berberis vulgaris extract (1, 7, and 30 mg/ mL) for 24 hours on WEHI-3 (murine myelomocytic leukemia cells), decreased the expression of p53 gene at all concentrations, with better results than the obtained with doxorubicin, a chemotherapeutic drug (SAEDI et al., 2015). This cytoplasmic protein, p53, is known to suppress tumorigenesis by acting on the regulation of G1 phase checkpoints allowing DNA repair or the removal of damaged cells through apoptosis. Mutations in p53 tumor suppressor gene are found in approximately 50% of all human cancers, leading the cell to replicate damaged DNA (HOLLSTEIN et al., 2015).

Wang et al. (2012) also showed cell cycle arrest on K562 leukemia cells exposed for 72 hours to 30 μg/ mL of a proanthocyanins solution from grape seeds. Grape seeds proanthocyanins reduced proliferation by induction of the cell cycle arrest in the G1 phase, the initial phase of the cycle preceding the S-phase of DNA doublin. Similarly, resveratrol exposure (200 μM for 24 hours) also resulted in induction of cell cycle arrest in G0/G1 phase...
and decreased the number of cells in S and G2 phases, indicating cell cycle arrest in four T-cell acute lymphoblastic leukemia cells, both glucocorticoid sensitive (CEM-C7-14) and glucocorticoid resists (MOLT4, JURKAT, CEM C7-15) types (GE et al., 2013). The mechanisms identified for the cell cycle arrest seems to be by the increase in p73 expression (p53 homologous protein) that can block G1 phase and trigger apoptosis when overexpressed (STRANO et al., 2000). In response to DNA damage, p73 is required to trigger the p53-dependent apoptosis mechanism (FLORES et al., 2002).

Oxidative stress may also play a role in many chronic diseases such as cancer. Garcia-Alonso et al. (2007) evaluated the ability of a phenolic-rich juice made from a mixture of grapes, cherries and other berries to protect human myelogenous leukemia cells (U937) from oxidative stress caused by tert-butyl hydroperoxide (tBOOH) (GARCIA-ALONSO et al., 2007). The results showed that pre-incubation of cells with the juice extract at 100μM for 3 hours provided protection against cell toxicity, DNA cleavage, and generation of intracellular oxygen reactive species. Deerberry extract also had high free radical scavenging capacity and anti-cancer properties, by inducing apoptosis, in human leukemia HL60 cell lines in a dose-dependent manner (30-120 μg/ mL) (WANG et al., 2007). Besides that, the study conducted by Katsuzaki et al. (2003) investigated the effects of anthocyanins isolated from skin of red grape (delphinidin 3-O-beta-D-glucoside, petunidin 3-O-beta-D-glucoside and malvidin 3-O-beta-D-glucoside) in human lymphoid leukemia MOLT4B cells. These researchers found that MOLT4B cells treated with anthocyanins showed typical morphological change of apoptosis like apoptotic bodies (1 mM anthocyanins for 72 hours) and fragmentation of genomic DNA after increasing concentrations of these anthocyanins from 0.2 to 1 mM for 72 hours. However, the antioxidant N-acetyl-L-cysteine suppressed the DNA fragmentation caused by anthocyanins, suggesting that reactive oxygen species (ROS) is involved in the induction of apoptosis in this experimental model. Polyphenol-rich black chokeberry (Aronia melanocarpa) juice (AMJ) containing predominantly chlorogenic acids, some cyanidin glycosides, and derivatives of quercetin and present a anticancer effect in the acute lymphoblastic leukemia Jurkat cell line, which is deficient for p53. AMJ induces early and late apoptosis by generating a pro-oxidant signal and triggering mitochondrial membrane potential loss with a subsequent release of cytochrome c. AMJ treatment also induced apoptosis of different human lymphoblastic leukemia cells (HSB-2, Molt-4 and CCRF-CEM), but not in normal lymphocytes (SHARIF et al., 2012).
Chemoresistance is one of the major obstacles to successful treatment of leukemia. One promising alternative is the identification of chemosensitive agents obtained from foods. This has been shown for some bioactive compounds, such as resveratrol that seems to have the ability to modulate drug-carrying proteins, such as P-glycoprotein (P-gp) and multidrug resistance protein (MRP1) (GUPTA et al., 2011). Increased expression of P-gp and MRP1 results in low accumulation of the drug in cells and has been implicated in the development of resistance to a variety of treatments (BARAN et al., 2007). Three cell lines of acute myeloid leukemia resistant to doxorubicin (AML2/DX100, AML2/DX100 and AML2/DX300) were treated with 50 μM of resveratrol for 72 hours. In this study, resveratrol was able to decrease regulation of the MRP1 gene and induce apoptotic death, suggesting that this bioactive compound may facilitate the effectiveness of doxorubicin (KWEON et al., 2010). Skupien et al. (2006) tested the effect of three different berry extracts (blueberry, raspberries and strawberries) against HL60 and two resistant cell lines (HL60/VINC and HL60/DOX). Among the extracts evaluated, blueberry at 0.240 g/ L for 72 hours was the most efficient against HL60 cells, but showed low activity against resistant cells. In contrast, raspberry (0.130 and 0.772 g/ L) and strawberry (0.133 g/ L and 0.609 g/ L) extracts exhibited high cytotoxic activity against HL60 and resistant cell lines at 72 hours.

The only study conducted in humans indicated a possible chemopreventive effect of blueberry/apple juice intake when evaluating oxidative or genotoxic risk biomarkers. After 4-weeks intervention of 1 L/ day of blueberry and apple juice, equivalent to 16 mg/ L of ascorbic acid and 97 mg/ L of quercetin an increase in plasma concentration of these compounds was observed as well as an anti-genotoxic effect and DNA oxidative damage protection (WILMS et al., 2007).

PART 2: EXPERIMENTAL

4.1.1 Main results

One strategy to overcome MDR is to identify compounds which can act selectively on MDR cells, a rare phenomenon known as collateral sensitivity (CS). In our laboratory we have demonstrated this effect with ellagic acid. We performed a cytotoxic assay on leukemia cells such as K562 (sensitive to drugs), Lucena-1 (MDR, expressing P-gp) and FEPS (MDR,
expressing P-gp and MRP1) (KOEFFLER & GOLDE, 1980; RUMJANEK et al., 2001). After 72 hours treatment with 25 µM of ellagic acid, the cytotoxicity was higher on FEPS (28.3 ± 3.3 % of viable cells) followed by Lucena-1 (75.3 ± 5.5% of viable cells) and it was not statistically significant on K562 (Figure 2A). The effect of ellagic acid after 96 hours of treatment was even higher on FEPS (26.3 ± 10.3% of viable cells) and Lucena-1 (64.0 ± 7.0% of viable cells) but it was not cytotoxic on K562 cells (Figure 2B). Therefore, both in 72 and 96 hours of exposure, ellagic acid showed to have cytotoxic effect on the most resistant cell line, FEPS, with no significant effect on sensitive cell line, K562. The results were confirmed by IC50 calculation with higher values for K562 and Lucena-1 cells and lower values for FEPS (Table 3). The identification of possible CS promoters could be effective in preventing MDR or making chemotherapy treatment efficient again.

**Figure 2:** The effects of ellagic acid on the cell viability of K562, Lucena-1 and FEPS cells. Cells were treated with different concentrations of ellagic acid for 72 (A) and 96 hours (B). The cells were plated at the final concentration of 2 x 10⁴ cells/ mL. Afterwards, were treated with different concentrations of ellagic acid.
dissolved in 0.25% of DMSO or 0.25% of DMSO (CTRL). After treatment, 20 μL of MTT were added, diluted in PBS, at final concentration of 0.5 mg/mL in each well. The plates were kept in an incubator at 37°C for 3 hours. Posteriorly, the plates were centrifuged at 200 g, for 7 minutes, the supernatant was discarded and the formazan crystals were dissolved in 200 μL of DMSO. The color intensity was measured in a microplate reader, with wave length of 492 nm. The percentage of cell viability was calculated as the ratio of treated cells to control cells. Data represent the mean ± SE of three independent experiments.

| Table 3: Cytotoxicity of ellagic acid (IC_{50}) in K562, Lucena-1 and FEPS cell lines. IC_{50} values were calculated from dose response curves. |
|-------------------------------|-----------------|-----------------|
| Cell line | 72 hours | 96 hours |
| K562 | 39.9 | 33.9 |
| Lucena-1 | 32.3 | 28.9 |
| FEPS | 17.4 | 17.9 |

5 CONCLUSIONS

Berries contain a complex mixture of phenolic compounds, such as ellagic acid, resveratrol, quercetin and anthocyanins. In vitro studies have shown that these fruits extracts, alone or in combination, as well as their isolated compounds may have a beneficial effect against leukemia. However, both animal models and clinical studies are still limited but crucial to consolidate evidence supporting the beneficial dietary dosage. Although some mechanisms have been identified, such as apoptosis induction, cell cycle arrest and induction of collateral sensitivity, its viability and efficacy in clinical studies are still unknown.

REFERENCES

ASOU, H. et al. Resveratrol, a natural product derived from grapes, is a new inducer of differentiation in human myeloid leukemias. Int. J. Hemat., v.75(5), p.528-533, 2002.

BARAN, Y. et al. Upregulation of multidrug resistance genes in doxorubicin resistant human acute leukemia cells and myelogeneous reversal of the resistance. Hematology, v. 12(6), p.511-517, 2007.

DAFLON-YUNES, N. et al. Characterization of a multidrug-resistant chronic myeloid leukemia cell line presenting multiple resistance mechanisms. Molecular and Cellular Biochemistry, v.383, p.123-135, 2013.

DAHLAWI, H. et al. Polyphenols are responsible for the proapoptotic properties of pomegranate juice on leukemia cell lines. Food Sci. Nut., v.1(2), p.196-208, 2013.

ESTEY, E. & DOHNER, H. Acute myeloid leukemia. Lancet, v.368, p.1894-1907, 2006.
FENG, R. et al. Cyanidin-3-rutinoside, a natural polyphenol antioxidant, selectively kills leukemic cells by induction of oxidative stress. *J. Biol. Chem.*, v.282 (18), p.13468–13476, 2007.

FERLAY, J. et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. *Int. J. Cancer*, v.136, p.E359–E386, 2015.

FIMOGNARI, C. et al. Induction of apoptosis in two human leukemia cell lines as well as differentiation in human promyelocytic cells by cyanidin-3-O-b-glucopyranoside. *Biochem Pharmacol.*, v.67(11), p.2047-2056, 2004.

FLORES, E. et al. P63 and p73 are required for p53-dependent apoptosis in response to DNA damage. *Nature*, v.416, p.560-564, 2002.

GARCIA-ALONSO, F. J. et al. Phenolic-rich juice prevents DNA single-strand breakage and cytotoxicity caused by tert- butylhydroperoxide in U937 cells: the role of iron chelation. *J. Nut. Biochem.*, v.18, p.457–466, 2007.

GE, J. et al. Resveratrol induces apoptosis and autophagy in T-cell acute lymphoblastic leukemia cells by inhibiting Akt/mTOR and activating p38-MAPK. *Biomed. Environ. Sci.*, v.26(11), p.902-911, 2013.

GUPTA, S. C. et al. Chemosensitization of tumors by resveratrol. *Ann. N Y Acad. Sci.*, v.1215, p.150-160, 2011.

GUTTERIDGE, J. M. & HALLIWELL, B. Free radicals and antioxidants in the year 2000: a historical look to the future. *Ann. N. Y. Acad. Sci.*, v.899, p.136-147, 2000.

HAGIWARA, A. et al. Ellagic acid, a natural polyphenolic compound, induces apoptosis and potentiates retinoic acid-induced differentiation of human leukemia HL-60 cells. *Int. J. Hematol.*, v.92, p.136–43, 2010.

HOLLSTEIN, M. et al. P53 mutations in human cancers. *Science*, v.5(253), p.49-53, 2015.

KATSUZAKI, H. et al. Cyanidin 3-O-beta-D-glucoside isolated from skin of black Glycine max and other anthocyanins isolated from skin of red grape induce apoptosis in human lymphoid leukemia Molt 4B cells. *Oncol Rep.*, v.10(2), p.297-300, 2003.

KOEFFLER, H. P. & GOLDE, D. W. Human myeloid leukemia cell lines: a review. *Blood*, v.56, p.344-350, 1980.
KWEON, S. H. et al. Resveratrol-mediated reversal of doxorubicin resistance in acute myeloid leukemia cells via downregulation of MRP1 expression. *Biochem. Biophys. Res. Commun.*, v.395, p.104–110, 2010.

MAIURI, M. C. *et al.* Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nat. Rev. Mol. Cell. Biol.*, v.8, p.741-752, 2007.

MERTENS-TALCOTT, S. U. *et al.* Low concentrations of quercetin and ellagic acid synergistically influence proliferation, cytotoxicity and apoptosis in MOLT-4 human leukemia cells. *J. Nutr.*, v.133, p.2669–2674, 2003.

MERTENS-TALCOTT, S. U. & PERCIVAL, S. S. Ellagic acid and quercetin interact synergistically with resveratrol in the induction of apoptosis and cause transient cell cycle arrest in human leukemia cells. *Cancer Letters*, v.218, p.141–151, 2005.

NILE, S. H. & PARK, S. W. Edible berries: Bioactive components and their effect on human health. *Nutrition*, v.30, p.134-144, 2014.

NUNEZ, G. *et al.* Caspases: the proteases of the apoptotic pathway. *Oncogene*, v.17(25), p.3237-3245, 1998.

RUMJANEK V. M. *et al.* Multidrug resistance in tumour cells: characterization of the multidrug resistant cell line K562-Lucena 1. *Anais da Academia Brasileira de Ciências*, v.73, p.57-69, 2001.

SAEDI, T. A. *et al.* *Berberis vulgaris* fruit crude extract as a novel anti-leukaemic agent. *Journal of Biological Regulators and Homeostatic Agents*, v.2(2), p.395-399, 2015.

SEERAM, N. P. Berry fruits: Compositional elements biochemical activities and the impact of their intake on human health, performance, and disease. *J. Agric. Food Chem.*, v.56, p.627-629, 2008.

SHARIF, T. *et al.* Red wine polyphenols cause growth inhibition and apoptosis in acute lymphoblastic leukaemia cells by inducing a redoxsensitive up-regulation of p73 and down-regulation of UHRF1. *Eur. J. of Cancer*, v.46, p.983-994, 2010.

SHARIF, T. *et al.*, *Aronia melanocarpa* juice induces a redox-sensitive p73-related caspase 3-dependent apoptosis in human leukemia cells. *Plos One*, v.7(3), p.1-11, 2012.

SKUPIEN, K. *et al.* *In vitro* antileukaemic activity of extracts from berry plant leaves against sensitive and multidrug resistant HL60 cells. *Cancer Letters*, v.236, p.282–291, 2006.

STONER, G. D. & SEERAM, N. P. Berries and cancer prevention. Springer Science Bussiness Media, LLC 2011.
STRANO, S. et al. Physical and functional interaction between p53 mutants and different isoforms of p73. J Biol Chem., v.275(29), p.503-12, 2000.

STRATTON, M. R. Exploring the genomes of cancer cells: progress and promise. Science, v.25(6024), p.1553-1558, 2011.

SZYMANOWSKA, U. et al. Antioxidant, anti-Inflammatory, and postulated cytotoxic activity of phenolic and anthocyanin-rich fractions from polana raspberry (Rubus idaeus L.) fruit and juice—In vitro study. Molecules, v.23(1812), p.1-17, 2018.

WANG, M. et al. Monocytic differentiation of K562 cells induced by proanthocyanidins from grape seeds. Arch Pharm Res., v.35(1), p.129-135, 2012.

WANG, Y. S. et al. Antioxidant activity in lingonberries (Vaccinium vitis-idaea L.) and its inhibitory effect on activator protein-1, nuclear factor-KB, and mitogen-activated protein kinases activation. J. Agric. Food Chem., v.53, p.3156–3166, 2005.

WANG, S. Y. et al. Antioxidant activity of Vaccinium stamineum: exhibition of anticancer capability in human lung and leukemia cells. Planta Med., v.73(5), p.451-60, 2007. Epub 2007 Mar 29.

WHITE, E. & DIPAOLA, R. S. The double-edged sword of autophagy modulation in cancer. Clin Cancer Res., v.15, p.5308-5316, 2009.

WILMS, L. C. et al. Impact of multiple genetic polymorphisms on effects of a 4-week blueberry juice intervention on ex vivo induced lymphocytic DNA damage in human volunteers. Carcinogenesis, v.28(8), p.1800–1806, 2007.

YANG, Z. J. et al. The role of autophagy in cancer: therapeutic implications. Mol. Cancer Ther., v.10, p.1533-1541, 2011.

ZHAI, Y. Berry fruit value-added products for health promotion. Boca Raton, FL: CRC Press 2007; 448p.

SUPPLEMENTARY MATERIAL

Table - Conceptual map with the study question: What is the role of red fruits in leukemias?

| Terms                        | DeCS/MeSH*                              | Synonyms                                       |
|------------------------------|-----------------------------------------|------------------------------------------------|
| Leucemia de Células Pilosas | Leucemia de Células Pilosas/Leukemia, Hairy Cell | Reticuloendoteliose Leucêmica, Leukemias Hairy Cell |
| Leucemia L1210              | Leucemia L1210/Leukemia L1210           | L1210 Leukemia                                 |
| Leucemia L5178              | Leucemia L5178/Leukemia L5178           | Linfoma L5178                                   |
| **Leucemia Linfoide** | Leucemia Linfoide/ Leukemia, Lymphoid | Leucemia Linfócita, Leukemias Lymphoid, Lymphocytic Leukemia*, Leukemias Lymphocytic |
|------------------------|----------------------------------------|--------------------------------------------------------------------------------|
| **Leucemia P388**      | Leucemia P388 /Leukemia P388           | Leucemia P388D(1) P388 Leukemia                                                |
| **Leucemia Plasmocitária** | Leucemia Plasmocitária                | Leucemia Plasmocítica, Leucemia de Plasmócitos                                 |
| **Leucemia Induzida por Radiação*** | Leucemia Induzida por Radiação/ Leukemia, Radiation-Induced | Leukemias Radiation-Induced                                                     |
| **Leucemia Eritroblástica Aguda** | Leucemia Eritroblástica Aguda/ Leukemia, Erythroblastic, Acute | Doença de Di Guglielmo, Mielose Eritrêmica, Eritroleucemia, Leucemia Mieloide Aguda M6 Erythroleucemia |
| **Leucemia Mieloide** | Leucemia Mieloide/ Leukemia, Myeloid | Leucemia Granulócítica, Leucemia Mielócita, Leucemia Mielógena Myeloid Leukemia*, Myelogenous Leukemia* |
| **Leucemia Megacarioblástica Aguda** | Leucemia Megacarioblástica Aguda/ Leukemia, Megakaryoblastic, Acute | Leucemia Megacariócítica, Leucemia Mieloide Aguda M7, Leucemia Megacariócita Leukemia Myeloid Acute M7, Leukemias Megakaryocytic |
| **Leucemia Monocítica Aguda** | Leucemia Monocítica Aguda / Leukemia, Monocytic, Acute | Leucemia Monoblástica Aguda, Leucemia Mieloide Aguda M5 Leucemia Mieloide Tipo Schilling Leukemia Myeloid Acute M5, Monoblastic Leukemias Acute |

**Linfoma de Burkitt** | Linfoma de Burkitt/ BurkittLymphoma | Linfoma Africano, Leucemia de Células de Burkitt, Linfoma-
| Leucemia de Mastócitos | Leucemia de Mastócitos/Leukemia, Mast-Cell | Leucemia Mastocitária | Leukemias Mast Cell |
|------------------------|------------------------------------------|----------------------|---------------------|
| Leucemia Prolinfocítica Tipo Células B | Leucemia Prolinfocítica Tipo Células B/ Leukemia, Prolymphocytic, B-Cell | Leucemia Prolinfocítica de Células B, Leucemia Prolinfocítica B |
| Leucemia Linfocítica Granular Grande | Leucemia Linfocítica Granular Grande/ Leukemia, Large Granular Lymphocytic | Leucemia Linfocítica Granular Grande de Células Matadoras Naturais, Leucemia Linfocítica Granular Grande de Células T, Leucemia Linfocítica Granular de Células Grandes Tipo T | Leukemia LGL, Leukemia Lymphocytic Large Granular |
| Leucemia-Linfoma Linfoblástico de Células Precursoras | Leucemia-Linfoma Linfoblástico de Células Precursoras/ Precursor CellLymphoblasticLeukemia-Lymphoma | Leucemia Linfoblástica Aguda de Células T, Leucemia Linfocítica Aguda de Células T, Leucemia Aguda de Células T, Leucemia Linfocítica Aguda Tipo T, T-ALL, Leucemia de Células T Aguda, Linfoma-Leucemia Linfoblástica de Precursor T, Leucemia-Linfoma Linfoblástica de Células T Precursoras, Leucemia-Linfoma Linfoblástico de Células Precursoras-T | LeukemiaLymphoblasticAcute |
| Leucemia-Linfoma Linfoblástico de Células T Precursoras | Leucemia-Linfoma Linfoblástico de Células T Precursoras/ Precursor T-CellLymphoblasticLeukemia-Lymphoma | Leucemia Linfoblástica, Leucemia Linfoides Aguda, Leucemia-Linfoma Linfoblástica de Células Precursoras, Linfoma Linfoblástico, Leucemia Linfocítica Aguda | LeukemiaLymphoblastic |
| Leucemia Mieloide Crônica Atípica BCR-ABL Negativa | Leucemia Mieloide Crônica Atípica BCR-ABL Negativa/ Leukemia, Myeloid, Chronic, Atypical, BCR-ABL Negative | Leucemia Mieloide Crônica Atípica, BCR-ABL Negativa, Leucemia Mieloide Crônica Atípica, Leucemia Mieloide Filadélfia-Negativa, Leucemia Mieloide Negativa para Filadélfia, Leucemia Mieloide Filadélfia Negativo | Leukemia Myelogenous Ph1-Negative, Leukemia Myeloid Philadelphia Negative |
| Leucemia Mielomonocítica Juvenil | Leucemia Mielomonocítica Juvenil/ Leukemia, Myelomonocytic, Juvenile | Leucemia Mielógênia Crônica Juvenil | Leukemia Myeloid, Juvenile |
| Leucemia de Células B | Leucemia de Células B/Leukemia, B-Cell | Leukemia Lymphocytic Cell B (28031)/Leukemia, B-Cell (15689) |
|-----------------------|----------------------------------------|---------------------------------------------------------------|
| Leucemia Linfocítica Crônica de Células B | Leucemia Linfocítica Crônica de Células B/Leukemia, Lymphocytic, Chronic, B-Cell | Leukemia Lymphocytic Chronic B-Cell (245) |
| Leucemia Basofílica Aguda | Leucemia Basofílica Aguda/Leukemia, Basophilic, Acute | Leukemia Basophilic Acute (196) |
| Leucemia Eosinofílica Aguda | Leucemia Eosinofílica Aguda/Leukemia Eosinophilic Acute | Leukemia Eosinophilic Acute (796) |
| Leucemia-Linfoma de Células T do Adulto | Leucemia-Linfoma de Células T do Adulto/Leukemia-Lymphoma, Adult T-Cell | Leukemia-Lymphoma, Adult T-Cell (796) |
| Leucemia Aguda Bifenotípica | Leucemia Aguda Bifenotípica/Leukemia Biphenotypic Acute | Leukemia Bifenotípica aguda/Leukemia biphenotypic acute de células mistas |
| Leukemia Mieloide de Fase Acelerada | Leucemia Mieloide de Fase Acelerada/Leukemia Myeloid Accelerated Phase | Leukemia Mieloide de Fase Acelerada/Leucemia Mieloide de Fase Agressiva/Leucemia Mielógena Crônica de Fase Agressiva/Leucemia Mieloide Crônica de Fase Acelerada/Leucemia Mieloide Crônica de Fase Agressiva |
| Leucemia Mielógênica Crônica BCR-ABL Positiva | Leucemia Mielógênica Crônica BCR-ABL Positiva/Leucemia Mielógena Crônica BCR-ABL Positiva/Leucemia Mieloide Crônica/Leucemia Mielógène Crônica Mieloictica | Chronic myelogenous leukemia BCR-ABL Positive (539)/leukemia Myelocytic Chronic (24439)/Leukemia Myeloid |

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| Blood Cancer Type | Brazilian Term | English Translation |
|-------------------|----------------|---------------------|
| Chronic (24163) Leukemia Myelocytic Chronic (24439)/ | Chronic Leukemia Myelocytic Chronic (24439)/ |
| Leucemia Mieloide de Fase Crônica | Leucemia Mieloide de Fase Crônica/ Leukemia Myeloid Chronic-Phase | Leukemia Myeloid Chronic-Phase Leukemia, Myeloid, Chronic-Phase (3607) |
| Leucemia Mielomonocítica Aguda | Leucemia Mielomonocítica Aguda/ Leukemia Myelomonocytic Acute (3124) | Leukemia Myelomonocytic Acute (3124) |
| Leucemia Mielomonocítica Crônica | Leucemia Mielomonocítica Crônica/Leukemia, Myelomonocytic, Chronic | Leukemia, Myelomonocytic, Chronic (2001) |
| Leucemia Neutrofílica Crônica | Leucemia Neutrofílica Crônica | Leukemia Neutrophilic Chronic (5) |
| Leucemia Mieloide Aguda | Leucemia Mieloide Aguda | Leukemia Lymphocytic Acute (32899)/ Leukemia Myeloid Acute (51) |
| Leucemia-Linfoma Linfoblástico de Células Precursoras B | Leucemia-Linfoma Linfoblástico de Células Precursoras B | Precursor B-Cell Lymphoblastic Leukemia-Lymphoma (6) |
| Leucemia Prolinfocítica de Células T | Leucemia Prolinfocítica de Células T | Leukemia Prolymphocytic T-Cell (589) |
| Leucemia Promielocítica Aguda | Leucemia Promielocítica Aguda/ Leucemia Mieloide Aguda M3 | Leukemia Promyelocytic Acute (6732)/ leukemia myeloid Acute M3 (957) |
| Leucemia | Leucemia | Leukemia (179263)/ leukocytosis (7986) |
| English Term                  | Portuguese Term                      |
|------------------------------|--------------------------------------|
| Leucemia de Células T        | Leucemia de Células T                |
| Leukemia, T-Cell(119)/       | Leukemia Lymphocytic Cells T (52)/   |
| Leucemia de Células T        | Lymphocytic (48)                     |

| Food Group                   | Frutas/fruits/                        |
|------------------------------|---------------------------------------|
| Antocianinas                 | Antocianinas/Anthocyanins             |
| Ácido elágico                | Ácido elágico/Ellagic Acid            |
| Flavonoides                  | Flavonoides/Flavonoids                |

*Related terms:* Segunda Neoplasia Primária, Vírus da Leucemia Induzida por Radiação.