

Original Article

Apoptosis induced by *Magnolia Grandiflora* extract in chlorambucil-resistant B-chronic lymphocytic leukemia cells

ABSTRACT

Background: B-cell chronic lymphocytic leukemia (B-CLL) still remains as an incurable disease. Even the newest antineoplastic agents have demonstrated limitations in their efficacy. For this reason, further research of new compounds must be done. New pharmacological properties can be obtained from a great diversity botanical species. Among these products, *Magnolia Grandiflora* receives our attention since it mainly contains Honokiol which had demonstrated effect against B-CLL cells activating different cell death pathways.

Aim: To test the ability of *Magnolia Grandiflora* extracts to induce apoptosis of B-CLL cells *in vitro*.

Materials and Methods: Herb's extraction: Twenty grams of powdered material were submitted to three consecutives decoctions with 500 ml of distilled water (96 °C), filtered and followed by ultrafiltration with cellulose membrane, lyophilized and reconstituted in AIM-V medium at a final concentration of 10 mg/ml solution. B-CLL chlorambucil-resistant cells were separated and cultivated in the presence of *Magnolia's* extract. Samples of cells were taken from the cultures at 24, 48 and 72 h for apoptosis analysis by flow cytometry measuring positive annexin V (0.1 µg/ml) cells. Statistics: Apoptosis values were represented by the mean plus or minus SD (\pm SD) for five independent experiments. Statistical significance was determined by Student's *t*-test. A *P* value of 0.05 or less was considered as significant.

Results and Conclusion: This article discusses the apoptosis properties of *Magnolia* on B-CLL cells. The evidence suggests a potentially effective repertoire for B-CLL treatment. This herb extract might have promising therapy strategies in treating B-CLL or other hematological disease resistant to alkylating agents in clinical practice.

KEY WORDS: Apoptosis, chronic lymphocytic leukemia, magnolia

INTRODUCTION

Diverse groups of botanical natural products have been described as the components of different Latin American Indians or Chinese traditional medicines.^[1,2] Anti-tumor properties of many of these compounds have been recognized. *Magnolia Grandiflora* mainly contains Honokiol, Magnolol and Parthenolide which is a sesquiterpene lactone.^[3] Honokiol and Magnolol are the major active constituents extracted from the bark of different Magnoliaceae species like *Magnolia officinalis* and *Magnolia Grandiflora*. They have a variety of pharmacological effects, such as anti-inflammatory,^[4] antithrombotic,^[5] anti-arrhythmic,^[6] antioxidant^[7] and anxiolytic effects,^[8] and more recently, cytotoxic activity by inducing cell apoptosis in some cell lines.^[9] Magnolol and Honokiol-triggered apoptotic process is accompanied with down-modulation of Bcl-XL molecules^[10] or through Caspase cascades activation.^[11] Parthenolide, a sesquiterpene lactone isolated from different herbs (included *Magnolia*),

is a novel NF-kappa B inhibitor^[3] with interesting antineoplastic properties.^[12-14] We previously had shown the anti tumor activity of Parthenolide in chronic lymphocytic leukemia (CLL) untreated model.^[15] Since Parthenolide is a component of *Magnolia*, this herb might also have antineoplastic properties.

The objective of this study has been to demonstrate *Magnolia Grandiflora's* capacity to induce apoptosis in untreated B-cell chronic lymphocytic leukemia (B-CLL) cells and chlorambucil-resistant B-CLL cells model.

MATERIALS AND METHODS

Herb's extraction: *Magnolia Grandiflora* seed cones were collected, dried and grounded. Twenty grams of powdered material was extracted in a glass pot by three consecutives decoctions with 500 ml of distilled water at the sub-boiling point (96°C.) for 50 min each, allowed to cool to room temperature, mixed, centrifuged and filtered through a microfilter

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followed by ultrafiltration with a cellulose membrane. The ultrafiltrates were lyophilized and then reconstituted for the experiment in AIM-V medium at a final concentration of 10 /CD23/CD19 in flow cytometry analysis (Coulter Epics XL).

Cell Cultures: Leukemia cells either B-CLL or chlorambucil-resistant B-CLL cells (ChR B-CLL cells-) were tested by seeding Magnolia's extract in triplicate 2 ml cultures at a cell density of 2×10^5 cells per culture in 15 ml Falcon test tubes and three different Magnolia's dilutions (1/300; 1/600 and 1/1200). The test tubes were incubated under standard conditions (37°C, 5% CO₂ and 80% humidity). Incubation was continued for two days. Samples of cells were taken from the cultures at 24, 48 and 72 h (by triplicate assays) for apoptosis analysis.

Apoptosis analysis: The percentage of CD5-CD19 cells in apoptosis process was analyzed by flow cytometry measuring positive annexin V (0.1 µg/ml) cells (by triplicate). Results were expressed as the average values of these measurements. Same cells submitted only to media cultures (no Magnolia product in the culture) served as negative apoptotic controls.

Statistics: Apoptosis values were represented by the mean plus or minus SD (\pm SD) for three independent experiments of each culture. Statistical significance was determined by Mann-Whitney non-parametric test. A P value of 0.05 or less was considered as significant.

RESULTS

Aqueous Magnolia extract displayed apoptotic effect on untreated B-CLL cells and also heavy treated with chlorambucil B-CLL cells *in vitro*.

Data shows that single 1/300 Magnolia's extract dilution is active against chlorambucil-resistant B-CLL cells since these cells had very low viability after 24 h in Anexin-V flow cytometry tests .

When apoptosis process was measured, we found that Magnolia extract [Figure 1] at 1/300 dilution caused apoptosis in $58.4 \pm 7.6\%$ of the ChR B-CLL cells or $62.3 \pm 5.2\%$ non-resistant B-CLL cells at 24 h [Figures 2 and 3] and $87.6 \pm 4.1\%$ or $84.9 \pm 6.9\%$ respectively at 48 h of culture with Magnolia's extract at 1/300 dilution [Figure 1]. However, at 1/600 or 1/1200 Magnolia's dilution, the apoptosis was similar to control group (placebo's extract) [Figure 1].

Statistical significant differences were observed between ChR B-CLL or non-resistant B-CLL cells submitted to 1/300 dilution Magnolia's extract, compared to either placebo or 1/600, 1/1200 dilution extracts ($P=0.001$ in all cases).

DISCUSSION

Since large amount of research done on B-CLL during the past

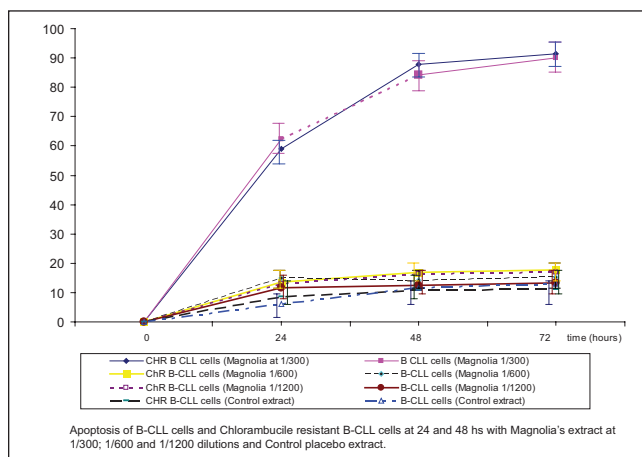


Figure 1: Apoptosis induced by Magnolia's extract at different dilutions

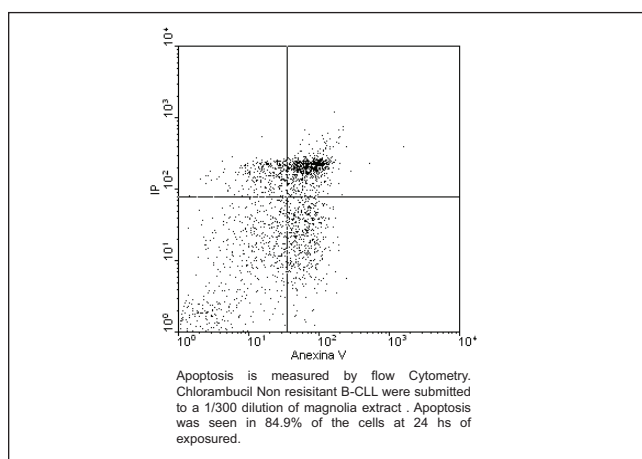


Figure 2: Apoptosis at 24 hs in ChR B-CLL cells induced by Magnolia's extract

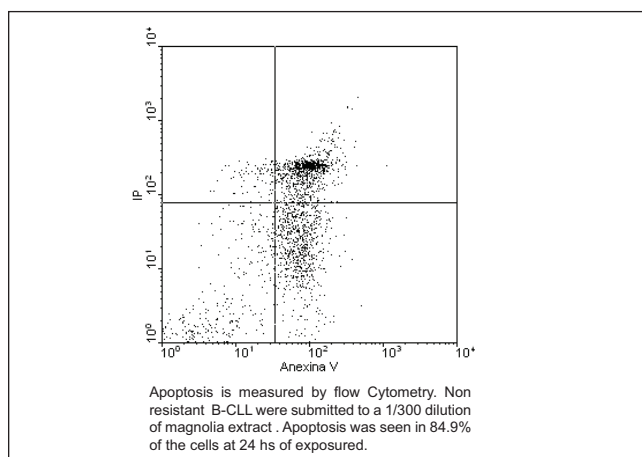


Figure 3: Apoptosis at 24 hs in non-resistant B-CLL cells induced by Magnolia's extract

years has not changed at all its prognosis, new therapeutic pathways are needed in order to change this illness outcome. This work shows the potent apoptotic effect of Magnolia

Grandiflora on B-CLL cells that had demonstrated previous resistance to chlorambucil treatment.

Our results provide clues of potentially interacting pathways involving different aspects of B-CLL cell apoptosis that could be exploited in future therapies. In previous papers,^[15] we had published that Magnolia's extracts have efficacy in apoptosis and cytotoxicity induction and that these properties are exhibited mainly in tumors and not in normal cells, suggesting that an increase in NF-kappa B inhibitory protein and a decrease in NF-kappa B DNA binding activity or EGFR/PI3K/Akt signaling pathway^[16] or inhibition of telomerase activity might be involved in apoptosis induction.^[17]

The water extract of Magnolia Grandiflora had demonstrated *in vitro* activity against both non-resistant and chlorambucil-resistant B-CLL cells suggesting that this family of herb's extracts might have promising therapy strategies in treating B-CLL and other hematological diseases resistant to alkylating agents.

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