Original Research Article
Annalen der Chemischen Forschung
Vol-1: No-2: 2013
www.aocsr.com

Sesquiterpene lactone extract from native american herbs demonstrated antineoplastic activity against Non Hodgkin Lymphoma cells

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Abstract

In the present work, the ability of Sesquiterpene lactone compound obtained from Magnolia extract and Parthenolide was studied for its ability to induce apoptosis of Follicular NHL cells in vitro. FNHL cells were obtained from stage IV patient, cultivated in presence of Magnolia's extract, Parthenolide, both compounds or AIM-V medium. Samples were taken from these cultures for apoptosis and cytotoxicity analysis by flow cytometry (annexin V) and trypan blue exclusion assay. Both Magnolia extract and Parthenolide have showed apoptosis and cytotoxic properties on NHL cells. This evidence suggests that SQL compounds extracted from common herbs like Magnolia might be promising therapy strategies in treating NHL illness.

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Key Words: Magnolia, Parthenolide, Apoptosis, Lymphoma

Cite this article as: Marin GH, Mansilla E, Ciocchini S, Quiroga N, Cardozo N, Weiss M , Gustavo H Marin ,Trebucq H. Sesquiterpene lactone extract from native american herbs demonstrated antineoplastic activity against Non Hodgkin Lymphoma cells. Annalen der Chemischen Forschung. 2013.;1(2):50-55.

1.0 Introduction

Sesquiterpene lactones (SQL) are composed by a sesquiterpenoids group (built from three isoprene units) containing a lactone ring. SQL are found in many plants and natural products used by Latin American Indians and Chinese traditional medicine [1,2]. Plants that are very common in Buenos Aires, Argentina contain different levels of sesquiterpene lactone group [3]. Plants like *Magnolia Grandiflora* contains primary active ingredients- Honokiol and Magnolol both compounds with high levels of SQL. Magnolia's extract has also very low levels of Parthenolide that has SQL as well as other two compounds. Sesquiterpene lactones compounds have a variety of pharmacological effects, such as anti-inflammatory [4], antithrombotic [5], anti-arrhythmic [6], antioxidant [7] or anti-leukemia effects [8, 9]. But there are few reports on apoptosis property of *Mangolia grandiflora*. Hence, In order to validate SQL's anticancer properties, we have screened the antineoplasic activity of seed cones *Magnolia Grandiflora* and one of its components alone –*Parthenolide*-, against atypical lymphocytes from follicular non Hodgkin lymphoma (NHL cells).

2.0 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 ºC.) for 50 minutes each, allowed to cool to room temperature, mixed, centrifuged and filtered though a microfilter followed by ultrafiltration with a cellulose membrane. The ultrafiltrates were lyophilized and then reconstituted for the experiment in AIM-V medium at final concentration of 10mg/ml solution, and used in the same day at serial dilution from 1/100 to 1/1000 when used alone.

Parthenolide: Since Magnolia extract has principally Honokiol and Magnolol and less amount of Parthenolide, this last compound was tested by itself. Parthenolide (Sigma) was reconstituted in dimethyl sulfoxide (DMSO) to a stock concentration of 0.2 M and further diluted in culture medium up to 10 to 1 μM with the final DMSO concentration <1%.

NHL cells: NHL cells samples were from bone marrow of patient with Follicular NHL stage IV. Cells were separated from heparinized peripheral blood by Ficoll-Hipaque density sedimentation and used for all experiments and controls respectively. Purity of NHL cells was determinate by the co-expression of CD10/CD19 in Flow Cytometry analysis (Coulter Epics XL).

Cell Cultures: NHL cells were tested by seeding *Magnolia*'s extract in 2 milliliter cultures at a cell density of 2 x 10^5 cells per culture in 15 milliliter Falcon test tubes with Magnolia's dilution (1/600). On the other hand, *Parthenolide* was added with cell seeding at 6 uM concentration. Also NHL cells were incubated with both *Magnolia'*s extract (1/600) and *Parthenolide* (6 uM) put them together. Finally cells were also tested just for culture medium (AIM-V medium) with

Apoptosis Analysis: The percentage of CD10-CD19 cells in apoptosis process was analyzed by flow cytometry measuring positive annexin V (0.1 μg/ml) cells (triplicate). Results were expressed as the average values of these measurements. Same cells submitted only to media cultures (neither Magnolia nor Parthenolide product in the culture) served as negative apoptotic controls.

Cytotoxic Analysis: percentages of viable cells were calculated by counting the living cells in relation to the total number of cells, using 0.4% trypan blue exclusion assay. Trypan blue (Gibco) was store in dark bottle and filter until usage. An aliquot of

cell suspension was centrifuged for 5 min at 100 ×g. Cell pellet was re-suspended in 1 ml pbs. 5 $\times 10^5$ of those cells were then diluted again by mixing 1 ml pbs. Mixture of 20 µl each of cell suspension and the same amount of 0.4% trypan blue was incubated by 3 min at room temperature. Cell death analysis was performed counting the total number of viable and nonviable cells using a binocular microscope according the following formula:

Viable cells(%)= (total number of viable cells per ml of aliquot/ total number of cells per ml of aliquot) x 100

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

Follicular Non Hodgkin Lymphoma cells in culture were expose to

1. Magnolia (1/600)
2. Parthenolide (6 uM)
3. Magnolia & Parthenolide (1/600 & 6 uM)
4. Control (AIM-V medium + DMSO)

All culture test tubes were incubated under standard conditions (37ºC, 5% CO_2 and 80% humidity). All incubations were continued for 2 days. Samples of cells were taken from the cultures at 24, 48 and 72 hours for apoptosis analysis (by triplicate assays). Cells were also tested for culture medium alone with and without 1% DMSO. Samples of cells were taken from the cultures at 0, 24, 48 and 72 hours and the in vitro cytotoxic as well as the apoptotic effect of PTL was measured by flow cytometry (Annexin V). Percentages of viable cells were also calculated by counting the living cells in relation to the total number of cells, using the trypan blue exclusion assay.

Product	AP 0 hs	CT 0 hs	AP 24 hs	CT 24 hs	AP 48 hs	CT 48 hs	AP 72 hs	CT 72 hs
Magnolia extract	0,1	0,3	31,3	15,3	61,4	32,7	80,4	53,5
Parthenolide	0,04	0	38,3	18,9	71,2	40,1	92,2	69,1
Magnolia & Parthenolide	0	0,1	39,3	21,1	79,8	41,8	94,7	78,2
Control	0,07	0	4,5	1,3	12,6	6,7	18,2	10,4

AP=Apoptosis, CT= Cytotoxicity,

Table 1: Apoptosis and Cytotoxicity of diferents SQL compounds

3.0 Results and discussion

The percentage of CD10+/CD19+ cells obtained from bone marrow patient's samples was 63±3.54% and after Ficoll-Hipaque density sedimentation process; purity of samples for CD10+/CD19+ cells was 90±2.41%. Both *Magnolia* extract and *Parthenolide* compounds had displayed apoptotic and cytotoxic effect on NHL cells in vitro. Data showed that 1/600 Magnolia`s extract dilution were active against NHL cells since these cells had shown low viability after 24 hours of culture in Anexin-V flow cytometry tests and cytotoxicity assay (Table-1).

Cytotoxic and apoptosis process were also measured in NHL cell samples exposed to Parthenolide compound and to the mix of Mangolia extract and Parthenolide (table 1). Both assays demonstrated high levels of apoptosis and toxicity if they were compared with control sample (figure 1). Statistical significant differences were observed between treated NHL cells submitted to Magnolia's extract, Parthenolide or mix of both products

compared with control medium+DMSO incubations (p 0.001 in all cases).

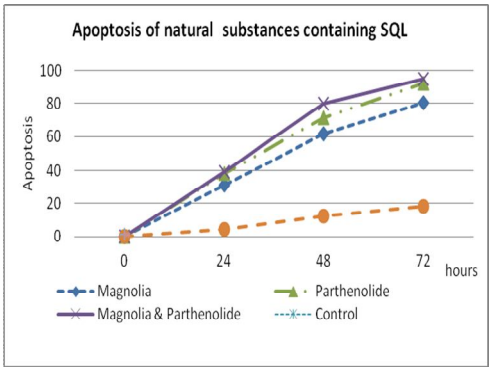


Figure-1

Apoptosis caused by SQL substances according to days in culture

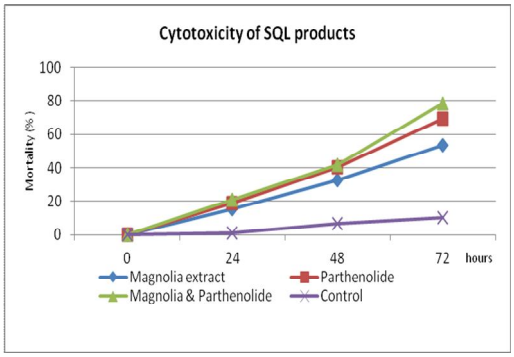


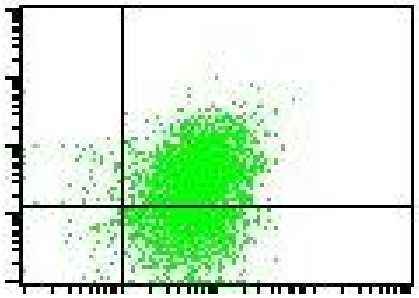
Figure-2

Cytotoxicity caused by SQL substances during the first 3 days in culture

These responses for NHL treated cells were time dependent since a significant decrease in cell viability was seen after one day of culture reaching more that 80% in all samples but controls at 72 hours. By contrast, control culture had little apoptotic or cytotoxic effect in NHL cells (table 1; figure 1). In previous papers [10], we had published that Magnolia's extracts have efficacy in apoptosis and cytotoxicity induction and that these properties are mainly in tumors and not 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 [11] or inhibition of telomerase activity might be envolved in apoptosis induction [12]. Also substances extracted from Magnolia Grandiflora like Magnolol and Honokiol-triggered apoptotic process by down-modulation of Bcl-XL molecules [11] or through Caspase cascades activation [13]. Parthenolide, the other sesquiterpene lactone substance analyzed in this paper could be isolated from different herbs (included Magnolia), is a novel NF-kappa B inhibitor [14] with could show interesting antineoplastic properties [15,16]. Our group previously demonstrated that either Magnolia extract or Parthenolide had shown an anti tumor activity against lymphocyte malignancies model [8-10].

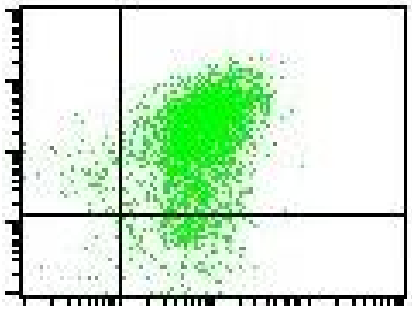
Thus, large amount of research was done in NHL during the last past years with these products showing that new therapeutic pathways could help in this

illness outcome. However it is interesting to demonstrate that sesquiterpene lactone sources like Magnolia extract and Parthenolide have also activity against other lymphocyte malignant pathologies like follicular lymphoma.



Apoptosis is measured by flow Cytometry. NHL cells were submitted to a 1/600 dilution of magnolia extract. Apoptosis was seen in 80.4% of the cells after 72hs of exposure.

Figure-3: Apoptosis at 72 hs in NHL cells induced by Magnolia's extract



Apoptosis data measured at 72 hs by flow Cytometry for NHL cells when these cells were submitted to 6 uM Parthenolide cultures . Apoptosis was seen in 92.2% of the cells after 72hs of *exposure.*

Figure-4: Apoptosis at 72 hs with Parthenolide

4.0 Conclusion

This work shows that Magnolia Grandiflora extract and Parthenolide has a potent apoptotic and toxic effect also on NHL Folicular lymphoma cells in vitro. Our results provide then clues of potentially interacting pathways involving different aspects of B-NHL-lymphocyte's apoptosis that could be exploited in future therapies.

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