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A fraction rich in phenyl propanoids from *L. divaricata* aqueous extract is capable of inducing apoptosis, in relation to H_2O_2 modulation, on a murine lymphoma cell line

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ABSTRACT

Leukemia and lymphoma are a group of heterogeneous neoplastic disorder of white blood cells characterized by the uncontrolled proliferation and block in differentiation of hematopoietic cells. Nowadays, there is an interest in therapy with drugs of plant origin because conventional medicine can be inefficient or also results in side effects. *Larrea divaricata* Cav., is a plant widely distributed in Argentina that possess antiproliferative and antioxidant activities reported. Nordihydroguaiaretic acid (NDGA) was previously found in the plant and related to both antiproliferative and pro-proliferative actions on a lymphoma cell line. In order to demonstrate whether the presence of NDGA may be beneficial or not in the antiproliferative action of the aqueous extract, the extract of *L. divaricata* was submitted to a fractionation and fractions with and without NDGA were studied in a murine lymphocitic leukemia cell line (EL-4) proliferation. The effect of the most active fraction was studied in relation to H_2O_2 modulation and the synergistic action between compounds, found in fractions, was analyzed. The presence of NDGA was not a detonator for pro-proliferative action and its presence could be beneficial in low concentrations allowing a synergist antiproliferative action with other compounds.

1. Introduction

Leukemia and lymphoma are a group of heterogeneous neoplastic disorders of white blood cells characterized by the uncontrolled proliferation and block in differentiation of hematopoietic cells. Meanwhile lymphoma is characterized by abnormal lymphocytes proliferation and appears as a solid tumor most commonly in the lymph nodes of the neck, chest, armpit or groin, leukemia does not [1]. According to the WHO, leukemia and lymphomas stand at the fifth place of cancer related death over the world [2]. Treatment of these pathologies includes radiotherapy, chemotherapy, hormonal therapy, immune therapy and symptomatic and supportive therapy. Nowadays, there is an interest in therapy with drugs of plant origin because conventional medicine can be inefficient or also results in side effects [3].

Plants have been recognized as the most important sources which might have numerous therapeutic agents. *Larrea divaricata* Cav. is a plant widely distributed in Argentina that presents antiproliferative and antioxidant activities reported [4,5]. Previously, it was demonstrated that an aqueous extract obtained from the leaves of the plants exerts both proliferative and antiproliferative action on a murine lymphoma cell line named BW 5147. Meanwhile the antiproliferative effect is related to the modulation of both hydrogen peroxide (H_2O_2) and nitric oxide (NO), the proliferative one, shown at low concentrations of the extract, is related to leukotrienes modulation [6]. Furthermore, it was shown that the same extract induces proliferation in a murine lymphocitic leukemia cell line (El-4) related to the presence of nordihydroguaiaretic acid (NDGA) [7]. The presence of nordihydroguaiaretic acid (NDGA) was previously described in the plant [4].

In order to demonstrate whether the presence of NDGA may be beneficial or not in the antiproliferative action of the aqueous extract, the extract of *L. divaricata* was submitted to a fractionation and fractions with and without NDGA were studied in a murine lymphocitic leukemia cell line (EL-4) proliferation. Moreover, since reactive oxygen species (ROS) can modulate normal and tumoral cell proliferation by inducing cell death in association with DNA damage, mutations and genetic instability [8], the effect of the most active fraction on the inhibition of cell proliferation was studied in relation to H_2O_2 modulation. Furthermore, the synergistic action between compounds, found in the most active fraction, was analyzed.

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2. Materials and methods

2.1. Plant material and preparation of extract

Leaves of *L. divaricata* Cav. were collected in the province of Cordoba, Argentina and identified using morphological, anatomical and histochemical analysis. A voucher specimen was deposited in the Museum of Pharmacobotany, School of Pharmacy and Biochemistry, University of Buenos Aires.

An aqueous crude extract of the leaves was prepared as follows: air-dried leaves were extracted with boiling distilled water, heated for a further 45 min at 56 °C with mechanical agitation and let to rest during 72 h at 5 °C. The extract was filtered, centrifuged, sterilized through a 0.22 μm membrane, lyophilized and stored at −20 °C until use [9]. After this, the aqueous extract was extracted with solvents twice overnight, firstly with dichloromethane (DM) and secondly with ethylacetate (EA). Finally three extracts were obtained DM, EA and Aqr (residual aqueous). The extracted were evaporated until dried residues were obtained. The residues were re-dissolved in distilled aqueous or in absolute ethanol to be studied upon cell proliferation.

2.2. Identification and quantification of compounds by HPLC

2.2.1. NDGA quantification

The amount of NDGA present in different fractions was determined by HPLC. The HPLC analysis was performed in a Varian Pro Star Instrument equipped with a Rheodyne injection valve (20 μl) and Photodiode array detector set at 280 nm. A reversed-phase column Phenomenex – C18 (2) Luna (250 mm × 4.6 mm and 5 μ dp) was used. The mobile face A was: water and acetic acid (98:2), B: Methanol and acetic acid (98:2). The gradient was from 15% B to 40% B in 30 min; 40% B to 75% B in 10 min; 75% B to 85% B in 5 min and 100% B in 5 min, leave 10 min 100% B and back to initial conditions. The mobile phase was delivered with a flow rate of 1.2 ml/min. Pure standard of NDGA (Sigma Lot 53H7090) was dissolved in methanol and water (70:30)

2.2.2. Quercetin-3-methyl ether identification and quantification

The identification of the peak with retention time 41 min present in EA fraction was performed by HPLC analysis. Three different methods were used by changing the mobile phase. A photodiode array detector set at 260 nm was used. (I) Method 1: the same described in Section 2.2.1. (II) Method 2: the mobile phase used was water: methanol: phosphoric acid (100:100:1). (III) Method 3: mobile phase: (A) water and phosphoric acid (99.5:0.5) and (B) methanol and phosphoric acid (99.5:0.5). The gradient was from 100% A to 25% A in 30 min, 25% A–0% A in 2 min and back to the initial conditions. The retention time of each method and the UV spectra were compared with a pure quercetin-3-methyl ether standard [10].

2.3. Cell suspensions and culture conditions

The lymphoma cell line EL-4 (ATCC) is a murine lymphocitic leukemia firstly induced by 9,10-dimethyl-1,2-benzanthracene in C57BL/6N mice. Cells were cultured at optimal concentrations of 5×10^5 cells/ml in RPMI 1640 medium (Gibco, NY, USA) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine and antibiotics and 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma, USA). Cells were settled at a final volume of 0.2 ml in 96-well flat-bottom microtiter plates for microculture [7].

2.4. Proliferation assays and viability studies

The effect on proliferation was evaluated by MTT method. Briefly, cells (3×10^5 cells/ml) were incubated alone or in presence of EA, DM, Aqr (from 0.01 to 1000 μg/ml). By other way, cells were incubated with EA 100 μg/ml alone or in presence of different inhibitors such as apocinin 10, 100 and 200 μM (NADPH oxidase inhibitor), L-NAME 6×10^{-4} M (inhibitor of nitric oxide synthase (iNOS), sodium azide 2×10^{-3} M (inhibitor of peroxidase) (Sigma) during 24 h. Then 100 μl of RPMI 1640 containing 10 μl of 5 mg/ml MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (Sigma) was added during at least 3 h. The purple formazan formed was solubilized by the addition of acidic isopropanol. The absorbance was measured using a microplate reader (Microplate Reader Benchmark BioRad) at 570 nm. Untreated cells were used as control and results were expressed as proliferation (%). Also this method was used to determine the synergistic antioproliferative effect between NDGA and quercetin-3-methyl ether.

On the other hand, the same method was used to determine cell viability but in this case cells were countered and the same quantity (10^6 cells) was incubated with MTT. Control cells, without any treatment were considered to be 100% viable so results were expressed as % of viability relative to control [11].

2.5. Apoptosis assays

To determine whether EA induced apoptosis, 3×10^5 tumoral cells were incubated with EA 100 μg/ml during 24 h. Cells were then washed twice with PBS, re-suspended in binding buffer at a concentration of 1×10^6 cells. Aliquots of 1×10^5 cells were incubated with annexin V-FITC (Sigma) and propidium iodide (Sigma), for 15 min at room temperature in the dark. Samples were analyzed by flow cytometry within 1 h. A control of apoptosis was done, incubating the cells in the same conditions but without FCS during 48 h [11].

2.6. Production of superoxide

The method described by Schopf [12] was employed where the O_2^- anion was evaluated by the reduction of nitroblue tetrazolium (NBT) (Sigma) to formazan. Briefly, tumoral lymphocytes were incubated with EA (100 μg/ml) and then with NBT during 30 min. The reaction was stopped with HCl. Formazan was extracted with dioxane and the absorbance was measured in a microplate reader at 525 nm.

2.7. Release of hydrogen peroxide (H_2O_2)

After treatment with EA, the determination of H_2O_2 was carried out by incubating tumoral lymphoma cells (3×10^5 cells/ml) with a solution of diaminobencidine tetrahydrochloride (DAB) containing type II horseradish peroxidase (Sigma). After 1 h incubation the reaction was stopped by the addition of NaOH and the absorbance was measured at 650 nm in a microplate reader. Results were expressed as nM of $H_2O_2/10^6$ cells. A standard curve of known molar concentrations of H_2O_2 in buffered phenol red was run in each test [13].

2.8. Total nitrite determination

The effect of EA upon total nitrites production by EL-4 cells was determined using the Griess reagents [14]. Briefly cells were incubated with or without EA 100 μg/ml for 24 h. After incubation, culture supernatants were collected and centrifuged, and then incubated with the Griess reagent for 20 min in the dark and measured at 540 nm. Total nitrites were calculated by interpolation in a standard curve made with known concentrations of nitrites.

2.9. Superoxide, catalase and peroxidase activity assays

2.9.1. Cells suspensions

Cells (2×10^6 cells/ml) were incubated 24 h in absence or absence of EA. After the addition of PMSF (Sigma) and Triton X 100 (Sigma) these cells were disrupted by pippeting and then centrifuged at $1500 \times g$ during 15 min. After centrifugation the supernatant was used for the determination of enzymatic activity.

2.9.2. Oxygen reducing activity: superoxide dismutase (SOD) activity

Fifty microliters of each cell supernatant (to determine SOD activity in cells) were diluted with sodium phosphate buffer (pH 10.7), and adrenaline (Sigma) were added immediately before the beginning of the OD monitoring at 480 nm kinetic mode. Results were expressed as units (U) of SOD activity/ml, where 1 U of SOD induces the inhibition of the auto-oxidation of adrenaline by a 50% [15].

2.9.3. Hydrogen peroxide scavenging activity: peroxidase (Px) activity

Supernatants were diluted with Krebs–Henseleit buffer (pH 7.4). The peroxidase activity was determined by the method described by Herzog and Fahimi [13]. Briefly, 50 μl of each sample were incubated with 3′, 5′ diaminobencidine tetrahidrochloride (DAB) (Sigma) and H_2O_2 in a final volume of 1 ml. A DAB solution without H_2O_2 was used as reaction blank. In all cases the reaction was initiated by the addition of H_2O_2 and the change in OD readings was recorded at 30 s intervals for 5 min using a Shimadzu recording spectrophotometer UV-240 (graphic printer PR-1) set at 465 nm. The Δ absorbance/min was calculated. A calibration curve of peroxidase concentration vs. Δ absorbance/min was lotted using horseradish peroxidase obtaining a linear relationship in the range of 1.95×10^{-3}–2.5×10^{-5} U/ml. The activity of samples was calculated by interpolation in the standard curve.

2.9.4. Hydrogen peroxide scavenging activity: catalase (CAT) activity

Catalase activity of samples was assessed by following the rate of disappearance of H_2O_2 with a spectrophotometer at 240 nm set in the kinetic mode. The incubation mixture was prepared diluting 0.1 ml of sample cell supernatant in phosphate buffer, then hydrogen peroxide were added immediately before beginning absorbance determination. The absorbance was recorded during 5 min and plotted versus time. The initial rate of disappearance of hydrogen peroxide (absorbance/minute) was calculated from the initial linear part of the curve (45 s). An extinction coefficient of 0.0394 cm^2/nmol proposed by Nelson and Kiesow for H_2O_2 was used to calculate it concentration. The catalase activity was then calculated using the following conversion: one unit of catalase is the amount of enzyme required to decompose 1 mmol of hydrogen peroxide per min, at 25 °C and pH 7.0 [15]. Results were expressed as U/ml.

2.10. Statistical analysis

Data was analyzed by the Student's t test and one way ANOVA and Dunnett's test. Differences were considered significant when $p \leq 0.05$.

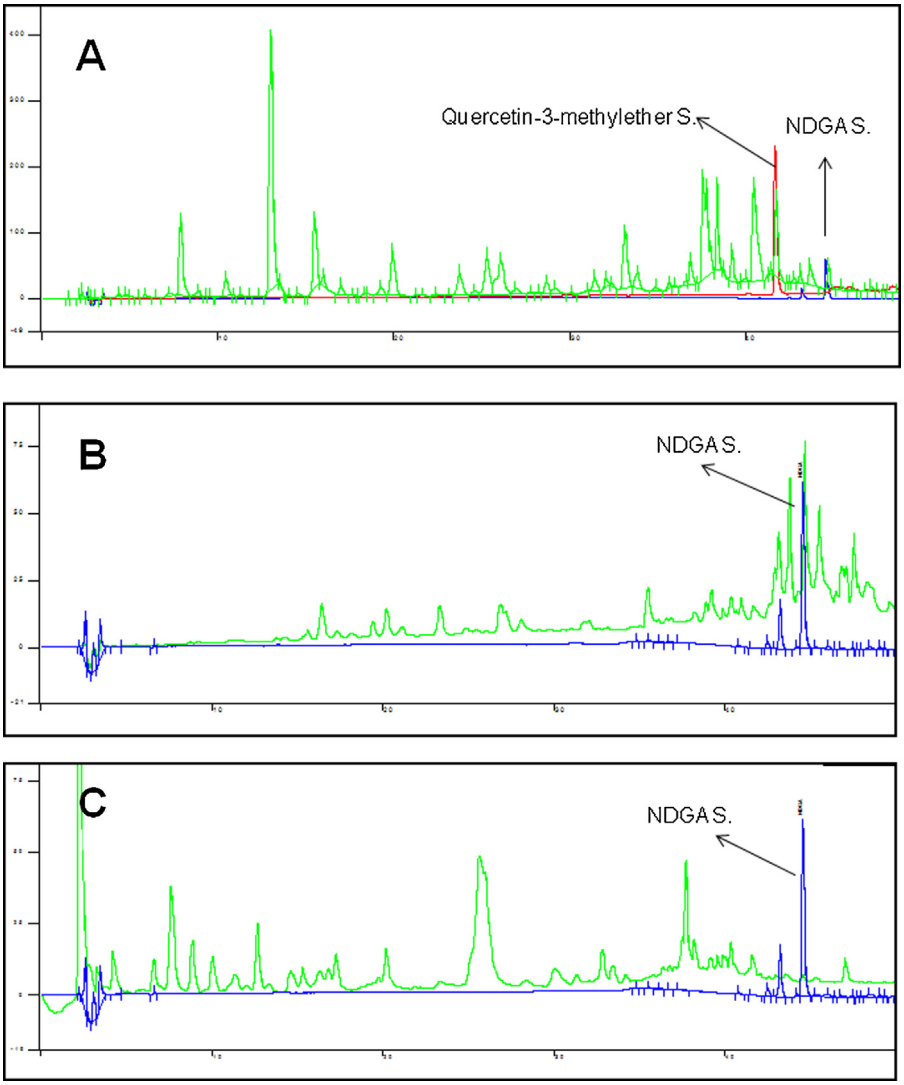


Fig. 1. HPLC analysis of fractions obtained from *L divaricata* aqueous extract. (A) Chromatogram of EA fraction and quercetin-3-methyl ether and NDGA standards. (B) Chromatogram of DM fraction and NDGA standard. (C) Chromatogram of Aqr and NDGA standard. The chromatograms are representative of three determinations. (S): Standard.

3. Results

Firstly, a fractionation of the aqueous extract was done in solvent of different polarity. Three fractions were obtained DM, EA and Aqr. The presence of NDGA and other compounds was determined by HPLC. DM and EA present different amount of NDGA in their composition whereas Aqr did not; also, other compound quercetin-3methyl ether was identified in EA.

DM present 0.56 g % of NDGA, EA 0.14 g % and crude aqueous extract between 0.6 and 0.7 g % (Fig. 1A and B).

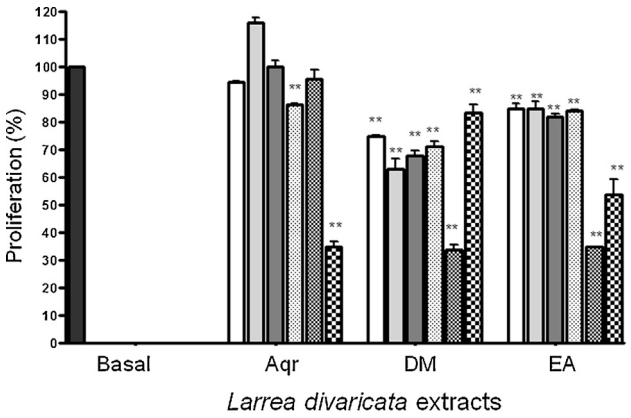
On cell proliferation, it can be seen in Fig. 2 that all the fractions exerted antiproliferative activity depending on the concentrations. DM presented the lowest EC_{50}, and the highest was observed with Aqr (Table inserted). Moreover, Aqr exerted a biphasic activity by increasing cell proliferation at low concentrations and inhibiting them at higher ones.

Since EA presents the lower NDGA quantities at the same time that exerted antiproliferative action it was selected to be studied on cell proliferation. EA decreased cell viability (Fig. 3A) at the same time that it induced cell apoptosis, principally late apoptosis (Fig. 3B and C).

When cells were incubated with EA alone or in presence of different inhibitors of the metabolism of ROS, it can be seen that, apocinin and L-NAME reversed EA effect meanwhile sodium azide increased it action (Fig. 4).

Furthermore, the effect of EA on enzymes related to H_2O_2 metabolism and NO level was studied. EA increased H_2O_2 at the same time that decreased $O_2^{\bullet -}$ and NO (Fig. 5A–C). By other way, decreased CAT, Px and SOD cell activities (Fig. 5D–F). To determine the participation of H_2O_2 in cell proliferation the effect of exogenous H_2O_2 was studied on EL-4 cell proliferation (Basal: 100% proliferation, H_2O_2 10^{-8} M: 125 ± 2.1; H_2O_2 10^{-5} M: 79 ± 7.4; H_2O_2 10^{-4} M: $19 \pm 1.72.1$).

Finally, NDGA and quercetin 3-methyl ether, the two compounds identified in EA, were studied in order to analyze their interaction and participation on EA antiproliferative activity. It can be seen in Fig. 6B that NDGA exerted antiproliferative action principally at high concentrations, meanwhile at low concentrations (0.32 μg/ml) increased it about 20%. Despite of this, it could decrease EC_{50} of quercetin-3-methyl ether for the antiproliferative action and also increased its maximum response. By other way, quercetin-3-methyl ether



Fraction	EC_{50} (μg/ml)
DM	12.88 ± 1.00
EA	15.48 ± 1.00
Aqr	288.00 ± 20.00

Fig. 2. Effect of fractions from *L. divaricata* aqueous extract on EL-4 proliferation. Cells were incubated with fractions in different concentrations (0.01–1000 μg/ml), during 24 h. Column white (0.01 μg/ml), column light gray (0.1 μg/ml), column dark gray (1 μg/ml), column with small squares (10 μg/ml), column with medium squares (100 μg/ml), column with large squares (1000 μg/ml). Table inserted: EC_{50} of the fraction on cell proliferation. Results were expressed as proliferation (% of basal) and represented mean ± SEM of three experiments made by triplicate.

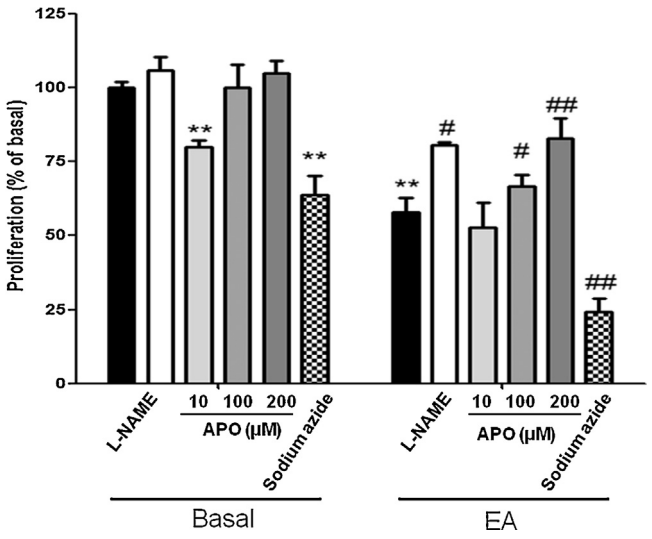


Fig. 4. Effect of inhibitors of ROS and NO on the antiproliferative action of EA on EL-4. Cells were incubated without (basal cells) (black column) or in presence of EA 100 μg/ml alone (black column) or in presence of Apocinin 10 μM, (light gray column), apocinin 100 μM (gray column), apocinin 200 μM (dark gray column), L-NAME (white column) and sodium azide (column with squares) during 24 h. Results were expressed as proliferation (% of basal) and represented mean ± SEM of three experiments made by triplicate. $^{*}p < 0.05$, $^{**}p < 0.01$ respect to basal; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ significantly differences between EA alone and in presence of inhibitors in accordance with ANOVA + Dunnett's test.

exerted low antiproliferative action but decreased EC_{50} of NDGA (Fig. 6A).

4. Discussion

In this work it was demonstrated that the fractionation of an aqueous extract from *L. divaricata* leaves, in solvent of different polarity, allowed to obtain a fraction with low concentration of NDGA, which exerted antiproliferative activity conducing cells to apoptosis. Moreover, this fraction exerted its activity by increasing H_2O_2 level and NO. The effect on H_2O_2 level was related to the modulation of enzymes which participated in H_2O_2 synthesis and metabolism.

Firstly, fractions with and without NDGA were obtained. The presence of NDGA was previously reported in *L. divaricata* [4,16]. In addition, it was shown that an aqueous extract of the plant

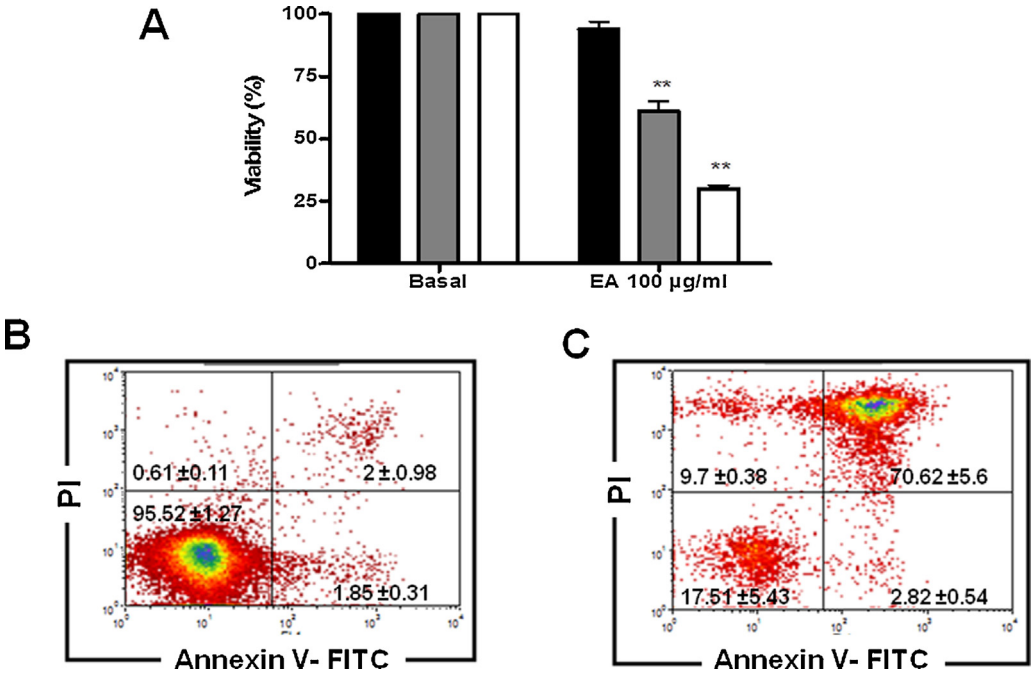


Fig. 3. Effect of EA on cell viability (A) and apoptosis (B and C). (A) Cells were incubated with EA (100 μg/ml), during 6 h (black column), 12 h (gray column) and 24 h (white column). Results were expressed as cell viability (% of basal) and represented mean ± SEM of three experiments made by triplicate. (B and C) Representative density plots analysis of basal control cells and cells treated with EA 100 μg/ml during 24 h. The percentage on each quadrant represented the cell in different stage of apoptosis: lower left: viable; lower right: early apoptosis; upper left: necrosis; upper right: late apoptosis.

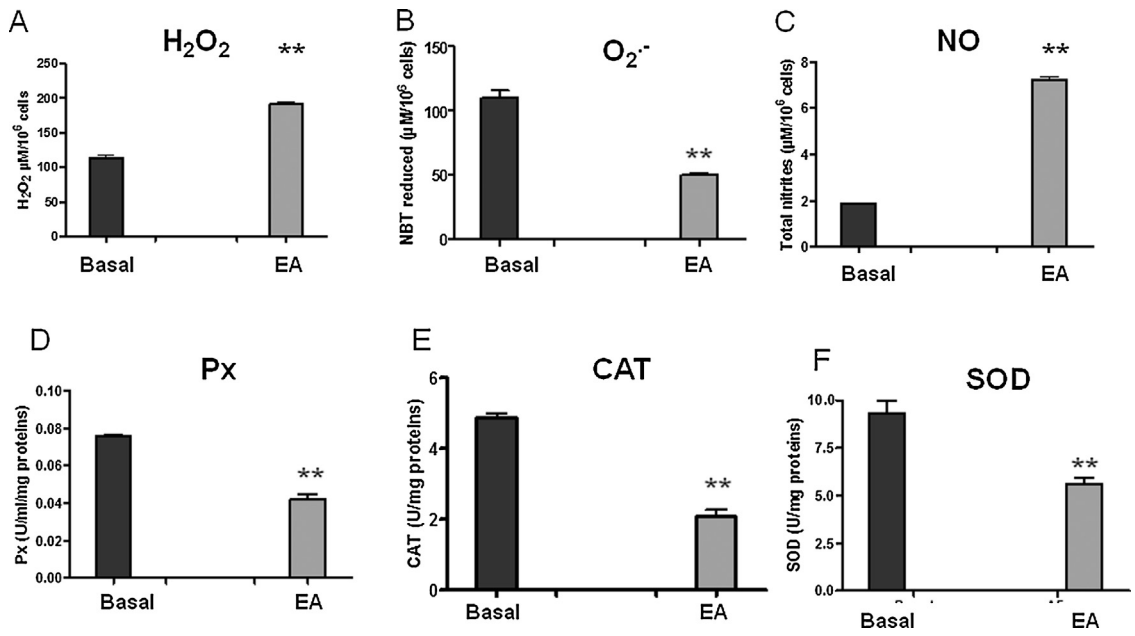
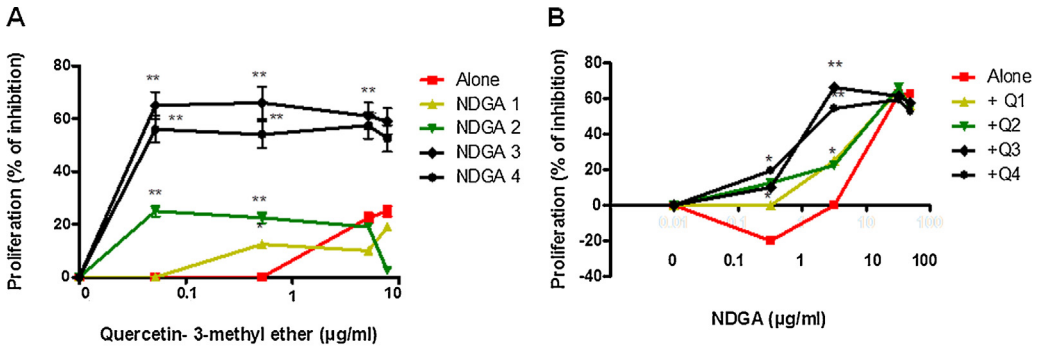


Fig. 5. Effect of EA on ROS production and on antioxidant enzymes activities. Cells were incubated without (basal cells) or in presence of EA 100 µg/ml during 24 h. Results represented mean ± SEM of three experiments made by triplicate. *$p < 0.05$, **$p < 0.01$ respect to basal in accordance with ANOVA + Dunnett's test.

exerts pro-proliferative action on EL-4 cells probably in relation to NDGA presence [7]. In that work pure NDGA, exogenously added to cell culture, induced cells to proliferate. Although DM presents the highest amount of NDGA, it exerted antiproliferative action. Since NDGA in the extract was not alone other compounds could contribute to DM antiproliferative activity. Also, EA, that present low NDGA quantities, exerted antiproliferative action which was not significantly different from that observed with DM. This suggested that other compounds with antiproliferative actions could be present. The absence of NDGA neither allowed to obtain more activity nor it presence induced cell proliferation. Which is more, Aqr, the fraction that did not present NDGA, exerted a biphasic effect by stimulating cell proliferation at low concentrations and inhibiting them at higher ones, this last could suggest that other compounds with pro-proliferative action and different from NDGA could also be present. The presence of NDGA is controversial as it had been used as antioxidant in alimentary industry during some years but then was prohibited by FDA in relation to nefrotoxicity and hepatotoxicity observed with high concentrations (50–100 µM (15–30 µg/ml). It is important to note that, these concentrations were present in DM extract. In contrast, low concentrations of NDGA, between 1 and 10 µM (0.3–6 µg/ml), such as those present in EA, are related to beneficial properties, which could be useful for humans. Furthermore, at these low concentrations it could act as enzyme inhibitor, antimicrobial agent, as a protector from neurotoxicity and bladder toxicity [17], as anticancer and as agent with antimutagenic action [18]. Because of this, EA was selected for further studies. EA exerted antiproliferative action as well as it



EC_{50} (µg/ml)

Alone	+ NDGA 1	+NDGA 2	+NDGA 3	+NDGA 4
1.86	0.30	0.05	0.01	0.01

EC_{50} (µg/ml)

Alone	+ Q1	+Q2	+Q3	+Q4
10.11	4.90	5.60	0.84	0.65

Fig. 6. Effect of quercetin-3 methyl ether and NDGA upon cell proliferation "in vitro". Cells were incubated with quercetin-3methyl ether alone or in association with different concentrations of NDGA (A) or were incubated with NDGA alone or in presence of quercetin-3 methyl ether (B). Table inserted: EC_{50} values for the antiproliferative action. The concentrations used for each drug were (µg/ml): NDGA1: 0.32; NDGA2: 3.2; NDGA3: 32.2; NDGA4: 48.8. Q1: 0.052; Q2: 0.52; Q3: 5.2; Q4: 7.8. Results represented mean ± SEM of three experiments made by triplicate. *$p < 0.05$, **$p < 0.01$ significantly differences respect to drug alone in accordance with ANOVA + Dunnett's test.

decreased cell viability. The decrease in cell viability was related to an apoptosis phenomenon by inducing cells to late apoptosis and necrosis.

Since ROS are involved in the maintenance of the balance cell proliferation/cell death, it was studied if EA would be capable to modulate ROS, which are known to be involved in cells apoptosis. Firstly, it was demonstrated that apocinin, a NADPH oxidase inhibitor, reversed EA activity on cell proliferation in a concentration response manner. Furthermore, sodium azide, an inhibitor of Px enzyme related to H_2O_2 metabolism, synergized the action of EA. As well sodium azide inhibited basal cell proliferation, when it was associated with EA the inhibition of proliferation was higher (57% in comparison with sodium azide alone ($p < 0.001$) and with EA alone ($p < 0.001$) in accord with AVOVA + Newman–Keuls test.

It is important to note that, NADPH oxidase is involved in $O_2^{\bullet -}$ synthesis which in turn is converted to H_2O_2 by superoxide dismutase. Taken all together, it could be possible that EA increased H_2O_2 level. This result was lately confirmed. Not only EA increased H_2O_2 level but also decreased $O_2^{\bullet -}$ suggesting that this last ROS was converting to hydrogen peroxide. Despite of decreasing SOD activity, enzyme related to H_2O_2 synthesis, EA decreased Px and CAT activities, enzymes related to H_2O_2 metabolism, so these results reinforced the hypothesis that EA was acting by increasing H_2O_2 level. Which is more, H_2O_2 demonstrated to modulate cell proliferation as it could decrease (at high concentration) or increase (at low concentrations) cell proliferation. In conclusion the increase in H_2O_2 level induced cell inhibition in these cells. There are evidences that H_2O_2 is related to the malignant transformation of cells but can also sensitize cancer cells to death by apoptosis [19]. By other way, there are data which suggest that H_2O_2-generating drugs may be an efficient way of killing cancer cells, in this sense, the effect of various chemotherapeutic agents used in clinic is mediated in part by an increase in cellular levels of H_2O_2 [20]. Also, previously it was demonstrated in other lymphoma cell line named BW5147 that hydrogen peroxide modulate cell proliferation [11].

Moreover, EA not only increased NO level, but also when cells were incubated with L-NAME (a iNOS inhibitor), their proliferation increased, suggesting that it also can induce cell death by this pathway. It is also known that NO is related to apoptosis by inducing cytochrome c release through mitochondrial membrane potential loss with the activation of JNK/SAP and P-38 MAPK pathway, triggered by H_2O_2 [21,22].

In order to analyze the participation of the compounds found in EA, NDGA and quercetin-3-methyl ether, in the antiproliferative action of the hole extract, it was studied firstly the antiproliferative action of each compound alone and then in presence of increased and fixed concentrations of the other. NDGA exerted pro-proliferative action at low concentrations; this effect was previously observed, at the same concentration [7]. It is important to note that, the concentration of NDGA (3.2 μg/ml) present in 100 μg/ml of EA did not exert by itself antiproliferative action either quercetin 3-methyl ether (0.52 μg/ml, concentration present in 100 μg/ml of EA) but together exerted antiproliferative activity (by inhibiting cell proliferation about 25%). This means that their presence could be important to achieve EA antiproliferative effect. Also, it was demonstrated that quercetin 3-methyl ether was capable to exert a synergistc action on NDGA, effect that it was shown as a shift of the concentration response curve of NDGA to the left and consequently related to a decrease of its EC_{50}. Nevertheless, it is important to note that, EA could present other compounds which effects could be synergistic.

In agree with these results, phenylpropanoids compounds are related to antioxidant and antiproliferative activities. These compounds normally found in plants are known to play an important role in the protection of the cells from oxidative damage [23]. Moreover, flavonoids have just attracted great interest because of their anti-allergic, anti-cancer, anti-inflammatory, hepatoprotective, antithrombotic and anti-viral effects [24].

It can be concluded that, the presence of NDGA at least at low concentrations could be beneficial to the action of crude extract allowing other compounds to exert antiproliferative action. So, a fraction with low NDGA quantity could be a potential therapy for lymphoma and leukemia treatment.

Conflict of interest statement

No conflict of interest to disclose.

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Contributions. R.M. and C.A. designed and performed the experiments. V.S. and R.A. performed the phytochemistry experiments. All of the authors analyzed and interpreted the data. C.A. wrote the paper, and all of the other authors contributed to writing the paper.

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