# Pannarin inhibits cell growth and induces cell death in human prostate carcinoma DU-145 cells

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**In the course of our continuing search for new natural anticancer compounds for treatment and/or prevention of prostate cancer, our laboratory has focused its search on poorly investigated lichen metabolites, sphaerophorin, pannarin and epiphorellic acid-1. To this end, we treated DU-145, a cell line resembling the last stage of prostate carcinoma, with different concentrations (6–50 μmol/l) of these compounds for 72 h. Our data clearly evidenced that these lichen metabolites inhibit the growth of human prostate carcinoma DU-145 cells, but pannarin exhibits a higher effect. Our data show an induction of apoptotic death of advanced prostate cancer cells by sphaerophorin, pannarin and epiphorellic acid-1. In fact, a significant ($P<0.001$) increase in caspase-3 enzyme activity occurred in DU-145 cells treated with all lichen compounds at 12 and 25 μmol/l concentrations, correlated to a high DNA fragmentation, but without the disruption of the plasma membrane, as evaluated by the percentage of lactic dehydrogenase release. Alternatively, we found a low, but significant ($P<0.01$) lactic dehydrogenase release at higher concentrations (50 μmol/l), suggesting that in these experimental conditions sphaerophorin, pannarin and epiphorellic acid-1 induce necrosis in DU-145 cells, through the increase in reactive oxygen species generation. The experimental evidence is further confirmed by caspase-3 activity results, evidencing a reduction in the activity of this protease at a higher concentration, 50 μmol/l. *Anti-Cancer Drugs* 17:1163–1169 © 2006 Lippincott Williams & Wilkins.**

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## Introduction

Prostate cancer is the most commonly diagnosed cancer in men and continues to be a major problem in the developed world [1]. Conventional therapy to eradicate tumor cells, e.g. surgery, chemotherapy, radiotherapy and hormonal treatments, has resulted in prolonged survival and cure in some patients [2]. Relapse and metastases, however, occur frequently, and in general are unresponsive to conventional therapy. In normal prostate tissue, androgens regulate the growth and differentiation of epithelial cells. In early stages of prostate cancer, proliferation is increased by androgens and can be kept in check by various therapies aimed at either decreasing circulating androgens or by blocking the androgen receptor using antagonists. In advanced stages of prostate cancer, however, growth and development typically become refractory to androgen effects, and cells continue to grow unchecked [3]. Aggressive treatment of patients with combination chemotherapy usually results in severe side-effects and is rarely curative [4–6]. In fact, androgen-independent tumors also become resistant to a wide variety of cytotoxic drugs. Currently, there is no therapy that has been shown to prolong the survival of these patients [1]. Therefore, new therapeutic approaches are needed with the objective of overcoming tumor cell resistance and reducing drug-mediated toxicity. In this regard, many nutritive and non-nutritive phytochemicals with diversified biological properties have shown promising responses for the prevention and/or intervention of prostate cancer, e.g. several natural antioxidants have been reported to have a chemopreventive effect on cancer [7–12]. In fact, for many years, there has been much interest in compounds extracted from plants as possible active substances in medicine. In addition, epidemiologic studies support the potential role of phytochemicals in the prevention and treatment of prostate cancer [13], and studies indicate that diets rich in soy isoflavones, green tea and lycopene are associated with reduced prostate cancer incidence [14,15]. The use of natural substances offers the possibility of discovering new biological mechanisms, to obtain new active molecules, and studying their structure–function relationships in order to develop more active drugs and to avoid unwanted side-effects. In addition, this allows us to obtain, through simple extraction, complex chemical structures that otherwise would require a long and complicated synthesis. Therefore, in the course of our continuing search for new natural anticancer compounds

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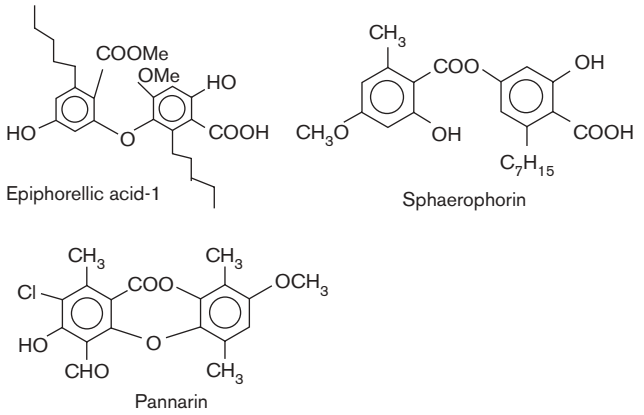
**Fig. 1**




Structure of lichen compounds, sphaerophorin (depside), pannarin (depsidone) and epiphorellic acid-1 (diarylether).

for the treatment and/or prevention of prostate cancer, the objective of this study was to assess the effects of poorly investigated lichen aromatic metabolites, sphaerophorin (depside), pannarin (depsidone) and epiphorellic acid-1 (diarylether) (Fig. 1), on DU-145 (androgen-insensitive prostate cancer cells), a cell line resembling the last stage of prostate carcinoma.

## Materials and methods

### Chemicals

3(4,5-Dimethylthiazol-2-yl) 2,5-diphenyl-tetrazolium bromide (MTT) and 2′,7′-dichlorofluorescein diacetate (DCFH-DA) were obtained from Sigma Aldrich (St Louis, Missouri, USA). All other chemicals were purchased from Gibco/BRL Life Technologies (Grand Island, New York, USA).

### Plant material

The tested metabolites arise from diverse lichen species collected in different localities of continental and arctic Chile.

Sphaerophorin, pannarin and epiphorellic acid-1 were isolated from *Sphaerophorus globosus*, different species of the genus *Psoroma* (*Psoroma reticulatum*, *P. pulchrum*, *P. palladium*) and *Cornicularia epiphorella*, respectively, as previously described [16,17]. The compounds, after extraction from all lichens, were isolated by chromatography using Si gel column and identified by spectroscopic techniques (infrared, $^{1}H$ and $^{13}C$ nuclear magnetic resonance, and mass spectrometry).

### Cell culture and treatments

Normal human prostatic epithelial cells were obtained from negative biopsies of human patients. DU-145 cells were obtained from the American Type Culture Collection (Rockville, Maryland, USA). The cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 µg/ml streptomycin, 1 mmol/l glutamine and 1% non-essential amino acids. After 24 h incubation at 37°C under a humidified 5% carbon dioxide to allow cell attachment, the cells were treated with different concentrations of lichen compounds (6–50 µmol/l) and incubated for 72 h under the same conditions. Stock solution of the natural compounds was prepared in dimethylsulfoxide (DMSO) and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone.

### MTT bioassay

MTT assay was performed as previously described [18]. Briefly, the cells were set up $6 \times 10^3$ cells per well of a 96-well, flat-bottomed 200-µl microplate. Cells were incubated at 37°C in a humidified 5% $CO_2$/95% air mixture and treated with lichen compounds at different concentrations (6–50 µmol/l) for 72 h. Doxorubicin (15 µmol/l) was used as a positive control. Four hours before the end of the treatment time, 20 µl of 0.5% MTT in phosphate-buffered saline was added to each microwell. Cells were washed once before adding MTT. After 4 h of incubation at 37°C, the supernatant was removed and replaced with 100 µl of DMSO. The optical density of each well sample was measured with a microplate spectrophotometer reader (Digital and Analog Systems, Rome, Italy) at $\lambda = 550$ nm.

### Lactic dehydrogenase release

Lactic dehydrogenase (LDH) activity was spectrophotometrically measured in the culture medium and in the cellular lysates at $\lambda = 340$ nm by analyzing nicotinamide adenine dinucleotide (reduced form) reduction during the pyruvate–lactate transformation, as previously reported [19]. Cells were lysed with 50 mmol/l Tris–HCl + 20 mmol/l ethylenediaminetetra acetic acid (EDTA), pH 7.4 + 0.5% sodium dodecyl sulfate, further disrupted by sonication and centrifuged at 13 000 *g* for 15 min. The assay mixture (1 ml final volume) for the enzymatic analysis contained 33 µl of sample (5–10 µg of protein) in 48 mmol/l phosphate-buffered saline, pH 7.5 plus 1 mmol/l pyruvate and 0.2 mmol/l nicotinamide adenine dinucleotide (reduced form). The percentage of LDH released was calculated as the percentage of the total amount, considered as the sum of the enzymatic activity present in the cellular lysate and that in the culture medium. A Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan) was used.

### Reactive oxygen species assay

Reactive oxygen species (ROS) determination was performed by using a fluorescent probe DCFH-DA, as previously described [19]. DCFH-DA diffuses through the cell membrane; it is enzymatically hydrolyzed by

intracellular esterases and oxidized to the fluorescent 2′,7′-dichlorofluorescein (DCF) in the presence of ROS. The intensity of fluorescence is proportional to the levels of intracellular oxidant species. An aliquot of 100 µl of 100 µmol/l DCFH-DA, dissolved in 100% methanol, was added to the cellular medium in which the acetate group is not hydrolyzed and the cells were incubated at 37°C for 30 min. After incubation, cells were lysed and centrifuged at 10 000*g* for 10 min. The fluorescence (corresponding to the radical species-oxidized DCF) was monitored spectrofluorometrically using a Hitachi F-2000 spectrofluorimeter: excitation 488 nm and emission 525 nm. The total protein content, measured according to Bradford [20], was evaluated for each sample, and the results are reported as fluorescence intensity/mg protein and are compared with relative control.

#### Activity of caspase-3

The activity of caspase-3 was determined by using the Caspase colorimetric assay kit (Sigma). This assay measures the cleavage of a specific colorimetric caspase substrate, acetyl-Asp-Glu-Val-Asp *p*-nitroanilide. *p*-Nitroaniline (pNA) is released from the substrate upon cleavage by caspase. Free pNA produces a yellow color that is monitored by a Hitachi U-2000 spectrophotometer at $\lambda = 405$ nm. The caspase-3 activity was measured in cell lysates. The cell pellets were incubated at 4°C for 20 min with lysis buffer containing 50 mmol/l 1-piperazineethane sulfonic acid, 4-(2-hydroxyethyl)-monosodium salt, pH 7.4, 5 mmol/l 3[(3-cholamidopropyl)dimethylammonio]-propanesulfonic acid, 5 mmol/l 1,4 dithio-DL-threitol. The lysed cells were centrifuged at 16 000 *g* for 15 min at 4°C and the supernatants were analyzed immediately according to the analysis procedure described in the manufacturer's protocol.

The total protein content, used to reflect cell number and measured according to Bradford [20], was evaluated for each sample, and the results are reported as $OD_{405\,nm}$/mg protein and are compared with relative control.

#### DNA analysis by COMET assay

The presence of DNA fragmentation was examined by single-cell gel electrophoresis (COMET assay), according to Singh *et al.* [21]. Briefly, $0.8–1 \times 10^5$ cells were mixed with 75 µl of 0.5% low-melting agarose and spotted on slides. The 'minigels' were maintained in lysis solution (1% *N*-laurosil-sarcosine, 2.5 mol/l NaCl, 100 mmol/l $Na_2EDTA$, 1% Triton X-100, 10% DMSO, pH 10) for 1 h at 4°C, then denatured in a high pH buffer (300 mmol/l NaOH, 1 mmol/l $Na_2EDTA$, pH 13) for 20 min, and finally electrophoresed in the same buffer at 18 V for 45 min. At the end of the run, the minigels were neutralized in 0.4 mol/l Tris–HCl, pH 7.5, stained with 100 µl of ethidium bromide (2 µg/ml) for 10 min and scored using a fluorescence microscope (Leica, Wetzlar, Germany) interfaced with a computer. Software (Leica-QWIN) allowed us to analyze and quantify DNA damage by measuring (1) tail length, intensity and area, and (2) head length, intensity and area. These parameters are employed by the software to determine the level of DNA damage as: (1) the percentage of the fragmented DNA (TDNA) and (2) tail moment (TMOM) expressed as the product of TD (distance between head and tail) and TDNA.

#### Statistical analysis

Statistical analysis of results was performed by using one-way analysis of variance followed by Dunnett's post-hoc test for multiple comparisons with control. All statistical analyses were performed using the statistical software package SYSTAT, version 9 (Systat, Evanston Illinois, USA).

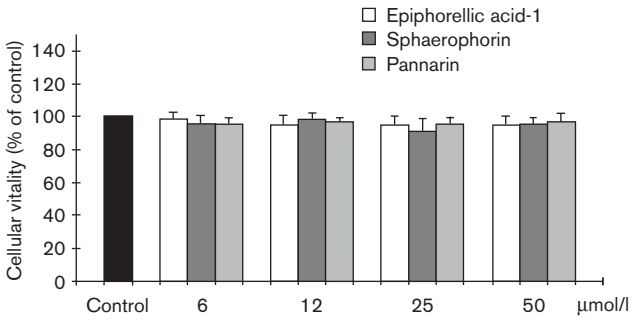
## Results

To evaluate the effects of lichen compounds on cell growth of human prostate carcinoma DU-145 cells, in this preclinical study *in vitro*, we cultured the cells in the absence or presence of sphaerophorin, pannarin and epiphorellic acid-1 at different concentrations (6–50 µmol/l), chosen on the basis of previous *in vitro* data on the same compounds [22,23] and on other natural compounds with hydroxyl groups in their structure [8,9,24], because pharmacokinetics studies for these lichenic metabolites are not present in the literature.

After treatment for 72 h, MTT assay, a non-radioactive assay widely used to quantify cell viability and proliferation, was performed. MTT is a yellow water-soluble tetrazolium salt. Metabolically active cells are able to convert the dye to water-insoluble dark blue formazan by reductive cleavage of the tetrazolium ring. The results show that our compounds at concentrations (6–50 µmol/l), non-toxic in normal human prostatic epithelial cells (Fig. 2), exhibited a significant inhibitory effect ($P < 0.001$) on DU-145 cell growth (Fig. 3). Pannarin exhibited the major effect. In fact, the vitality was 57, 31 and 20% in cells exposed to 25 µmol/l of epiphorellic acid-1, sphaerophorin and pannarin, respectively. Necrosis results in a disruption of cytoplasmic membrane, and the necrotic cells release cytoplasmic LDH and other cytotoxic substances into the medium. We therefore examined the membrane permeability of the treated cells and the existence of LDH in their culture medium. No statistically significant increase in LDH release was observed in DU-145 cells treated with lichen compounds at 12 and 25 µmol/l concentrations. Conversely, a low but significant increase in LDH was observed at higher concentrations (50 µmol/l) (Table 1).

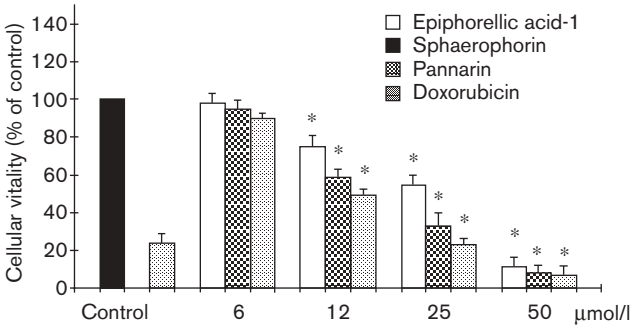
ROS have been reported to be involved in cell death induced by a variety of stimuli. We therefore examined

**Fig. 2**




Cell growth, assayed using the MTT test, of normal human prostatic epithelial cells untreated and treated with sphaerophorin (depside), pannarin (depsidone) and epiphorellic acid-1 (diarylether) at different concentrations (6–50 µmol/l) for 72 h. Stock solution of compounds was prepared in dimethylsulfoxide (DMSO) and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone. Each value represents the mean ± SD of three experiments, performed in quadruplicate.

**Fig. 3**



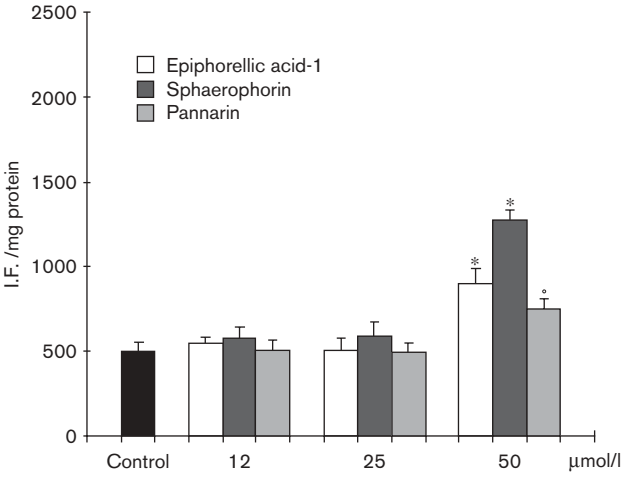

Cell growth, assayed using the MTT test, of DU-145 cells untreated and treated with sphaerophorin (depside), pannarin (depsidone) and epiphorellic acid-1 (diarylether) at different concentrations (6–50 µmol/l) for 72 h. Doxorubicin (15 µmol/l) was used as a positive control. Stock solution of compounds was prepared in dimethylsulfoxide (DMSO) and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone. Each value represents the mean ± SD of three experiments, performed in quadruplicate. *Significant vs. control untreated cells ($P<0.001$).

**Table 1 Lactate dehydrogenase (LDH) release in DU-145 cells untreated and treated with sphaerophorin (depside), pannarin (depsidone) and epiphorellic acid-1 (diarylether) at different concentrations (6–50 µmol/l) for 72 h**

| Treatments | % LDH released |
|---|---|
| Control | 3.9 ± 0.7 |
| Epiphorellic acid-1 (µmol/l) | |
| 12 | 3.3 ± 0.1 |
| 25 | 4.4 ± 0.6 |
| 50 | 7.5 ± 0.6* |
| Sphaerophorin (µmol/l) | |
| 12 | 4.9 ± 0.9 |
| 25 | 6.3 ± 0.7 |
| 50 | 7.5 ± 0.6* |
| Pannarin (µmol/l) | |
| 12 | 5.6 ± 0.7 |
| 25 | 3.4 ± 0.9 |
| 50 | 9.8 ± 0.9* |

Stock solution of compounds was prepared in dimethylsulfoxide (DMSO) and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone. The results are expressed as percentage of LDH released into the cell medium with respect to total LDH. Each value represents the mean ± SD of three experiments, performed in quadruplicate. *Significant vs. control untreated cells ($P<0.01$).

**Fig. 4**

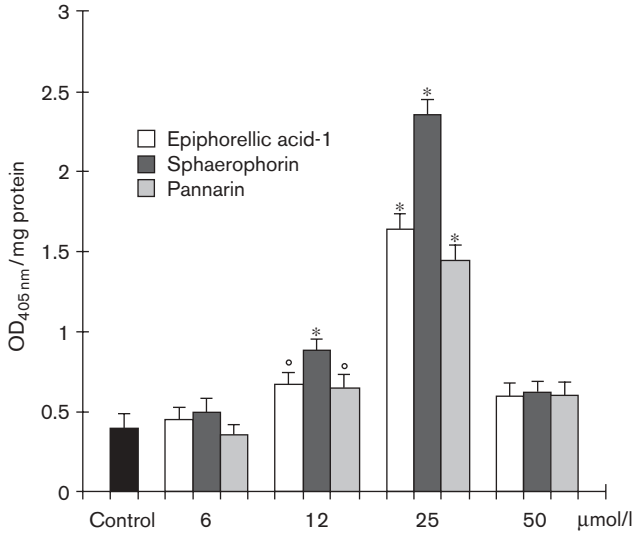



Reactive oxygen species (ROS) determination, performed by using a fluorescent probe 2′,7′-dichlorofluorescein diacetate, in DU-145 cells untreated and treated with sphaerophorin (depside), pannarin (depsidone) and epiphorellic acid-1 (diarylether) at different concentrations (6–50 µmol/l) for 72 h. Stock solution of compounds was prepared in dimethylsulfoxide (DMSO) and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone. Each value represents the mean ± SD of three experiments, performed in quadruplicate. *Significant vs. control untreated cells ($P<0.001$). °Significant vs. control untreated cells ($P<0.05$).

whether lichen compound-induced cytotoxicity could result from an elevation of ROS. To assess changes in intracellular ROS levels, we employed an oxidation-sensitive fluorescent probe DCFH-DA. DCFH-DA can be taken up into cells and then oxidized by ROS to its fluorescent derivative DCF. We found that the DCF fluorescence increased significantly only in cells treated with 50 µmol/l lichen compounds (Fig. 4).

Active caspases cleave several important intracellular proteins, leading to the morphological and biochemical changes associated with apoptosis, such as oligonucleosomal fragmentation of chromosomal DNA [25]. Caspase-3 is the major executioner caspase in the caspase cascade; therefore, the next experiments were performed to characterize the role of activation of this protein in cell growth inhibition mediated by lichen compounds. As shown in Fig. 5, the activity of caspase-3, measured by pNA, released from the specific caspase substrate and reported as $OD_{405\,nm}$/mg protein, was increased only in

**Fig. 5**




Caspase-3 activity, determined by using the Caspase colorimetric assay kit (Sigma), in DU-145 cells untreated and treated with sphaerophorin (depside), pannarin (depsidone) and epiphorellic acid-1 (diarylether) at different concentrations (6–50 µmol/l) for 72 h. Stock solution of compounds was prepared in dimethylsulfoxide (DMSO) and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone. Each value represents the mean ± SD of three experiments, performed in quadruplicate. *Significant vs. control untreated cells ($P<0.001$). °Significant vs. control untreated cells ($P<0.05$).

DU-145 cells treated with epiphorellic acid-1, sphaerophorin and pannarin at 12 and 25 µmol/l concentrations. Conversely, at 50 µmol/l concentration, the activity of this protease returned to the control value, also when the results obtained were referred to cell number (data not shown).

Nuclear DNA fragmentation was analyzed using the COMET assay, a sensitive method for detecting DNA strand breaks in individual cells and a versatile tool that is highly efficacious in human biomonitoring of natural compounds [26]. Quantification of the COMET data is reported as TDNA and TMOM in Table 2. The results clearly evidence DNA damage in cells exposed to all natural compounds examined for 72 h, but these lichen metabolites produced a drastic increase in both TDNA and TMOM at 12 and 25 µmol/l concentrations. These results seem to confirm apoptotic cell death, because recent data in the literature [27] indicate that only comets with high values of TMOM (tail moments) and TD (distance between head and tail of the comet) can be related to apoptosis.

## Discussion

Lichens are complex symbiotic organisms of fungi and algae. They are the earliest colonizers of terrestrial habitats on the Earth, and they show a worldwide

**Table 2 Comet assay of genomic DNA in DU-145 cells untreated and treated with sphaerophorin (depside), pannarin (depsidone) and epiphorellic acid-1 (diarylether) at different concentrations (6–50 µmol/l) for 72 h**

| Treatment | TDNA | TMOM |
|---|---|---|
| Control | 15 ± 3.5 | 97 ± 5.7 |
| Epiphorellic acid-1 (µmol/l) | | |
| 6 | 18 ± 5.3 | 118 ± 16 |
| 12 | 89 ± 17* | 635 ± 26* |
| 25 | 99 ± 15* | 964 ± 37* |
| 50 | 39 ± 4.5* | 366 ± 16* |
| Sphaerophorin (µmol/l) | | |
| 6 | 22 ± 7 | 124 ± 15 |
| 12 | 96 ± 11* | 1708 ± 21* |
| 25 | 99 ± 16* | 1899 ± 65* |
| 50 | 34 ± 5.5* | 418 ± 21* |
| Pannarin (µmol/l) | | |
| 6 | 21 ± 7.8 | 126 ± 7.5 |
| 12 | 84 ± 18* | 1706 ± 34* |
| 25 | 94 ± 15* | 1010 ± 51* |
| 50 | 43 ± 6.5* | 312 ± 25* |

Stock solution of compounds was prepared in dimethylsulfoxide (DMSO) and the final concentration of this solvent was kept constant at 0.25%. Control cultures received DMSO alone. The values are the mean ± SD of three experiments performed in quadruplicate. TDNA, the percentage of the fragmented DNA; TMOM, tail moment expressed as the product of TD (distance between head and tail) and TDNA. *Significant vs. control untreated cells ($P<0.001$).

distribution from arctic to tropical regions and from the plains to the highest mountains. In particular, they are the most conspicuous macroscopic organisms in Continental South America (Chile) and in Antartica, in terms of species, biomass and distribution [28]. Throughout the ages, lichen extracts have been used for various purposes, in particular, as dyes, perfumes and for various remedies in folk medicine since ancient Egyptian times [29]. Lichens and their metabolites have long been used by humans. Several studies on the secondary metabolites present in lichens, as well as on advances in understanding the chemistry of these metabolites, have previously been reported and have led to the isolation from all lichens, the fungus forms a thallus or lichenized stroma of many new lichen substances, which today number over 800 [28]. These secondary metabolites, which comprise aliphatic, cycloaliphatic, aromatic and terpenic compounds, are unique with respect to those of higher plants [28,29]. Usnic acid, the prototype of low-molecular-weight compounds derived from lichens, has been the most extensively studied and is used in pharmaceutical preparations [30]. Usnic acid acts as a commercial preparation against infections, bacterial eczema, mastitis, furunculosis and polydermy. In addition, preclinical studies have permitted us to hypothesize its possible use as a antineoplastic agent. A recent work of screening revealed [24] that usnic acid displayed cytotoxic activity in a dose-dependent manner in different cancer cells (L1210, 3LL, DU-145, MCF7, K-562 and U251).

Depsides and depsidones are two major and representative groups derived from polyketide pathway. The depsides are formed by condensation of two or more hydroxybenzoic

acids, whereby the carboxyl group of one molecule is esterified with a phenolic hydroxyl group of a second molecule. Depsidones have an ether linkage in addition to the ester linkage of the depsides, resulting in a rigid polycyclic system. Therefore, they are based on an 11*H*-dibenzo[*b*,*e*][1,4]dioxepin-11-one ring system. Depsides and depsidones, isolated from lichens, have been shown to have activity against mycobacteria, insects and nematodes [28]. Previously, our study evidenced antifungal and antibacterial activities of depside and depsidone metabolites such as epiphorellic acid-1 [31]. In addition, several well-characterized depsidones and depsides exhibit anti-inflammatory, analgesic, antipyretic and anticancer properties that, at least in part, can be correlated with their ability to scavenge free radicals and ROS [28]. Pannarin, like other lichen compounds, exhibited antioxidant activity in rat brain homogenate auto-oxidation and β-carotene oxidation, and the results obtained in both systems indicate that lichenic metabolites afford a protection in the μmol/l concentration range [32]. This study also evidenced that depsidones, and in particular pannarin, proved to be more efficient than depsides. The authors suggest that the higher effect of the depsidones could be related to a larger incorporation into lipid microdomains. Moreover, it is interesting to underline that it has been shown that bridging at the phenolic group in the *p*-position can increase the antioxidant activity of phenols due to a more efficient overlap of substituent orbitals within the aromatic π system [32].

Most of the presently available anticancer drugs mediate their effect via apoptosis induction in cancer cells and apoptosis is suggested as one of the major mechanisms for the targeted therapy of various cancers including prostate cancer. In case of advanced prostate cancer, cancer cells become resistant to apoptosis and do not respond to cytotoxic chemotherapeutic agents [33,34]. Therefore, the agents that induce apoptotic death of hormone-refractory prostate cancer cells could be useful in controlling this malignancy. Consistent with this approach, our data show an induction of apoptotic death of advanced prostate cancer cells by sphaerophorin, pannarin and epiphorellic acid-1. In fact, a significant increase in caspase-3 enzyme activity occurred in DU-145 cells treated with all lichen compounds at 12 and 25 μmol/l concentrations, correlated with a high DNA fragmentation, but without the disruption of the plasma membrane, as evaluated by the percentage of LDH release. Alternatively, we found a low but significant LDH release at a higher concentration (50 μmol/l), suggesting that in these experimental conditions sphaerophorin, pannarin and epiphorellic acid-1 start a necrotic pathway in DU-145 cells, through the increase of ROS generation (Fig. 4). Our hypothesis is further confirmed by caspase-3 activity results, evidencing a reduction in the activity of this protease at higher concentration, 50 μmol/l (Fig. 5). In fact, it has been reported that in some cell lines caspase inhibition can switch the mode of death from apoptosis to necrosis [35]. On the other hand, it has been found that the magnitude of the initial insult, rather than the type of stimulus, decides whether the cell dies through apoptosis or necrosis [36]. Studies reveal that apoptosis and necrosis are not necessarily independent pathways. Instead, they may share some common messengers, activators and inhibitors [37]. The central and novel finding in the present preclinical study, however, is the identification of major efficacy of pannarin, against advanced human prostate carcinoma DU-145 cells (hormone-refractory human prostate cancer). The data obtained in our experimental conditions evidenced that pannarin (depsidone) is more potent in inhibiting the growth of DU-145 as compared with sphaerophorin (depside) and epiphorellic acid-1 (diarylether). In fact, the growth-inhibitory effect of these agents in DU-145 cells is in the following order: pannarin (depsidone) > sphaerophorin (depside) > epiphorellic acid-1 (diarylether). Doxorubicin, eteposide and paclitaxel are the most commonly used chemotherapeutic agents in the treatment of metastatic hormone-refractory prostate cancer. The overall response rates induced by these drugs, however, are very poor and the duration of response is very short, with a limited impact on survival. In addition, the level of cytogenetic damage in peripheral blood lymphocytes of patients undergoing chemotherapy has been analyzed incisively and the results showed a high level of damage [38,39]. The results obtained in our experimental conditions have more value, considering that pannarin at the tested concentrations does not exhibit toxicity in human lymphocytes [22] and hepatocytes [23], and that also at higher concentrations, its toxicity effect was lower than in depsides.

In summary, as pannarin shows antioxidant activity [32], presents a low toxicity [22–23] and reduces the growth of DU-145 cells activating an apoptosis process, it is possible to hypothesize a possible use as a novel therapeutic agent for prostate cancer prevention or in an advanced stage of cancer in association with classic drugs with the aim to reduce their toxicity.

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