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Bryonia dioica aqueous extract induces apoptosis through mitochondrial intrinsic pathway in BL41 Burkitt's lymphoma cells

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ABSTRACT

Ethnopharmacological relevance: *Bryonia dioica* Jacq. is a climbing perennial herb with tuberous roots which is widely used in traditional medicine in Algeria for the treatment of cancers; it belongs to the genus Bryonia (Cucurbitaceae).
Aim of the study: To investigate the cytotoxic and apoptogenic activities, the phytochemical composition and acute toxicity of the aqueous extract of *Bryonia dioica* roots growing in Algeria.
Materials and methods: Dried roots of *Bryonia dioica* were extracted with water (decoction). The cytotoxic effects of the aqueous extract in the Burkitt's lymphoma BL41 cell lines were evaluated by flow cytometry. Apoptosis induction was assessed by two corroborative assays; propidium iodide (PI) staining of cell DNA and flow cytometric light scatter analysis. The mitochondria membrane potential was investigated using a fluorescent dye DIOC6. The expression of caspases-3, -8, -9 and PARP was assessed by Western blot. The phytochemical screening of the roots of *Bryonia dioica* was performed using qualitative phytochemical standard procedures.
Results: The *Bryonia dioica* aqueous extract induced cell death in a dose-dependent manner. The IC50 of *Bryonia dioica* aqueous extract was estimated to be approximately 15, 63 μg/ml. This was accompanied by induction of apoptosis, activation of caspase-3 and -9, cleavage of PARP and loss of mitochondria membrane potential. Furthermore, the phytochemical screening of roots of *Bryonia dioica* showed the presence of various bioactive such as polyphenols, sterols and triterpenes, alkaloids, c-heterosides, carbohydrates and saponins.
Conclusion: The aqueous extract of *Bryonia dioica* induces apoptosis in the Burkitt's lymphoma BL41 cell lines via the mitochondrial pathway. The flavonoids, sterols and triterpens detected could be responsible for the cytotoxic and apoptogenic activities of the aqueous extract of *Bryonia dioica*. These findings suggest that *Bryonia dioica* could be considered as a promising source for developing novel therapeutics against Burkitt's lymphoma.

1. Introduction

Plants have played a vital role in the prevention and treatment of disease since prehistoric times (Assefa et al., 2010). According to the WHO, it is estimated that 60% of antitumoral medicines are derived, either directly or indirectly, from medicinal plants (Robinson and Zhang, 2011). A large majority of anticancer drugs used currently, or still undergoing clinical trials, are derived from natural sources (Efferth et al., 2007; Lautiéa et al., 2008).

Bryonia dioica Jacq. a climbing perennial herb with tuberous roots which occurs in temperate Europe, North Africa, and western Asia (Sallam et al., 2010), belongs to the genus Bryonia in which some species may contain cytotoxic cucurbitacines (Frohne et al., 2009; Sallam et al., 2010). *Bryonia dioica*, is used for both internal and external uses (Leporatti and Ghedira, 2009). It is taken orally in small quantities for the treatment of various inflammatory conditions, bronchial complaints, asthma, intestinal ulcers, hypertension and arthritis. Externally, it is applied as a rubefacient to muscular and joint pains and pleurisy. It has been reported that the plant is used in folk medicine as a drastic purgative, emetic, bitter tonic and anti-diabetic agent (Matsuda et al., 2010). In different European countries it is considered to be a cicatrising agent (Guarrera and Lucia, 2007). *Bryonia dioica*, popularly named in

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Algeria "Fachira" and "queriou'âa" by the locals, is widely used in traditional medicine (Baba Aissa, 1999) to treat muscular–skeletal problems and cancer (Gonźalez-Tejero et al., 2008).

There is an abundance of medicinal plants in Algeria but many of these plants have yet to be investigated for their phytochemical and biological properties (Rached et al., 2010). This study was undertaken to determine the scientific basis for the traditional uses of *Bryonia dioica* as anticancer plant. The cytotoxic and apoptogenic activities of the aqueous extract of *Bryonia dioica* in Burkitt's lymphoma BL41 cell lines were investigated. In addition, the phytochemical composition was studied.

2. Materials and methods

2.1. Chemicals

Anti-caspase-3 (9662) and anti-caspase-9 (9502) antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Anti-caspase-8 (5F7) was obtained from Upstate Biotechnology (Lake Placid, NY, USA). Anti-PARP-1 was from BD Pharmingen (Franklin Lakes, NJ, USA). (TU-02) antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). Propidium iodide was from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Cells and culture conditions

The Burkitt's lymphoma BL41 cell line was provided by Dr. Aimé Vazquez (U1014, INSERM, Hopital Paul Brousse, Villejuif, France). The cells were cultured in RPMI 1640 medium with Glutamax supplemented with 10% FCS, 100 U/ml penicillin, 100 l g/ml streptomycin, sodium pyruvate, and 1% nonessential amino acids (Life Technologies, Strasbourg, France) in a humidified atmosphere with 5% CO_2 in air at 37 °C.

2.3. Preparation of Bryonia dioica aqueous extract

The roots of *Bryonia dioica* were collected in March, 2010 in Mascara, Algeria. Botanical identification and authentication was done by Dr. Kada Righi (Department of Agriculture, Faculty of Nature and Life sciences, Mascara University, Algeria). A voucher specimen of the plant (voucher number: SNV/B-2010/019) was deposited in the herbarium of the Department of Biology, Faculty of Nature and Life Sciences, Mascara University, Algeria. The collected roots were dried at room temperature, pulverized and finely sieved. The *Bryonia dioica* aqueous extract was prepared as follows: the dried roots were boiled for 20 min at 100 °C, cooled to room temperature, and then filtered. The filtered solution was collected, concentrated, lyophilized and stored in a desiccator at +4 °C until used.

2.4. Detection of apoptotic cells

2.4.1. Analysis of dot-blot light scatter profiles

Cells were seeded in 96-well plates at a density of 1×10^5 cells/ml and were incubated with *Bryonia dioica* aqueous extract (0–500 μg/ml) for 24 h. Apoptotic cells were detected by flow cytometry as described in Schrantz et al. (2001).

Cells were washed in phosphate-buffered saline (PBS), pelleted and resuspended in PBS. Their dotblot light scatter profiles were analyzed by flow cytometry using a FACScan flow cytometer (Accuri C6, Accuri Cytometers Inc., Ann Arbor, MI, USA). Apoptotic cells cause lower forward light scatter (caused by cell shrinkage) and higher side scatter (caused by increased granularity of the cell, presumably as a result of chromatin condensation and fragmentation) than their viable counterparts (Shan et al., 1998). Shrunken cells with relatively high side-scatter and low forward-scatter properties were considered to be apoptotic and enumerated as a percentage of the total population.

2.4.2. Hypodiploid DNA

Following exposure to *Bryonia dioica* aqueous extract for 24 h, BL41 cells (10^6) were washed in PBS and resuspended in 1 ml of hypotonic fluorochrome solution (50 μg/ml propidium iodide in 0.1% sodium citrate plus 0.1% Triton X-100) as described by Clybouw et al. (2005). Samples were placed at room temperature for 1 h before flow cytometric analysis of the propidium iodide fluorescence of individual nuclei using a FACScan flow cytometer (Accuri C6, Accuri Cytometers Inc., Ann Arbor, MI, USA). Debris was excluded from analysis by raising the forward scatter threshold. The DNA content of the intact nuclei was registered on a logarithmic scale. Apoptotic cells were identified on the basis of their nuclei having hypodiploid DNA, emitting fluorescence in channels 10–200. These cells were counted, and their number was expressed as a percentage of the total population (Clybouw et al., 2005).

2.5. Analysis of mitochondrial membrane potential

The loss of mitochondrial membrane potential was assessed by flow cytometry according to the method of El Mchichi et al. (2007). Briefly, $\Delta\Psi m$ was evaluated by staining cells (10^6) with DIOC6 at a final concentration of 40 nM (stock solution 40 mM in ethanol) for 15 min at 37 °C in the dark. The fluorescence emitted by cells was analyzed with a FACScan flow cytometer (Accuri C6, Accuri Cytometers Inc., Ann Arbor, MI, USA) using the fluorescence signal 1 channel. Cells with low $\Delta\Psi m$ were counted and their number expressed as a percentage of the total population.

2.6. Western blot analysis

Cytosolic protein extracts were prepared as described by Qi et al. (2010). Briefly, treated and untreated cells were collected by centrifugation at 300 × *g* for 5 min at 4 °C, washed with icecold PBS twice and lysed with RIPA lysis buffer (20 mM Tris–HCl buffer, pH 7.5, containing 1 mM EDTA, 1 mM DTT, 1 mM PMSF, and 1 mM Protease Inhibitor Cocktail) for 30 min on ice, and debris was removed by centrifugation at 10,000 × *g* for 10 min. Aliquots of the supernatants were used for protein determination by Bradford method. Equal amounts of protein (30 μg) were subjected to SDS-PAGE, and the proteins were then electrophoretically transferred onto nitrocellulose membranes. The blotted membranes were blocked for 1 h with 5% on fat milk in Tris-buffered saline, 0.1% Tween 20. The membranes were then incubated overnight at 4 °C with specific antibodies. Blots were washed three times for 10 min, in Tris-buffered saline, 0.1% Tween 20 and incubated for 1 h with peroxidase-labeled anti-mouse or anti-rabbit immunoglobulins. After washing three times in Tris-buffered saline, 0.1% Tween 20, images were captured using a DDC camera (LAS-1000; Fuji).

2.7. Phytochemical screening

The roots of *Bryonia dioica* were screened for the presence of phytochemical constituents such as alkaloids, terpenoids, anthraquinones, flavonoids, tannins, saponins, steroids and glycosides using qualitative phytochemical standard procedures described by Trease and Evans (1983) and Harborne (1998).

2.8. Statistics

All statistical comparisons were made by Student's *t*-test and statistical significance was defined as $p < 0.05$.