Potential antioxidant activity, cytotoxic and apoptosis-inducing effects of *Chelidonium majus* L. extract on leukemia cells

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Abstract

OBJECTIVES: The purpose of this study was to assess whether a methanol extract isolated from the greater celandine *Chelidonium majus* L. (CME) had antioxidant effect and was able to inhibit proliferation and to induce apoptosis in leukemia cells *in vitro*.

METHODS: The potential antioxidant activity of CME was proved by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The cytotoxicity of CME was measured by the cell growth inhibition assay using murine leukemia L1210 cell line and human promyelocytic HL-60 leukemia cells. Apoptosis-inducing effect was determined by fluorescence microscopy (chromatin condensation and nuclear DNA fragmentation).

RESULTS: In the DPPH assay CME acted as a scavenger of DPPH free radical. The results on antiproliferative properties assessment clearly demonstrated that CME had a cytotoxic effect towards both leukemia cell lines in a dose-dependent manner. In addition, the human promyelocytic HL-60 cells were more sensitive to CME treatment than the L1210 cells.

CONCLUSIONS: We concluded that the extract of *C. majus* L. had a strong antioxidant potential and exerted the antiproliferative activity via apoptosis on leukemia cells. CME due to the presence of the isoquinoline alkaloids and the flavonoid components may play an important role in both cancer chemoprevention through its antioxidant activity and modern cancer chemotherapy as cytotoxic and apoptosis-inducing agent.

ORIGINAL ARTICLE

Abbreviations

CME - extract from the greater celandine *Chelidonium majus* L.
DPPH - 1,1-diphenyl-2-picrylhydrazyl free radical
FRAP - ferric reducing and antioxidant power

INTRODUCTION

Many plant extracts are nowadays considered as a powerful alternative to some of the synthetic medications and are extensively used in treating a wide range of illnesses. Among natural substances there are many antioxidants and other bioactive compounds which may have an important function in protection against numerous diseases [5,9,13]. In connection with a high alkaloid content accounting for a broad variety of biological activities, *Chelidonium majus* L. has a multiple application in traditional folk medicine. It is because of its antitumoral, anti-inflammatory, anti-arthritic, antimicrobial activities and influence on diverse cellular processes [1,8].

Greater celandine (*Chelidonium majus* L.) is a plant of the Papaveraceae family, which is known for its richness in specific alkaloids. It is widely distributed in Europe and some parts of Asia growing on hedge banks, hedgegrows and walls and also often on wasteland. But it can be found also in North America or in Azores archipelago [1,2].

The plant contains many secondary metabolites, e.g., isoquinoline alkaloids such as sanguinarine, chelidonine, chelerythrine, coptisine or berberine. Caffeic acid derivatives, several flavonoids and phenolic acids are also present [2,7,17]. They possess a broad spectrum of various pharmacological activities. Their anti-tumor activity is conveyed through different mechanisms, which may be promising targets for anti-cancer therapy [6,7].

Over the last decade, considerable advances have been attained in the field of apoptosis-based therapeutic agents. The ability to induce apoptosis is a common mechanism of the cytotoxic effect of the most DNA-damaging drugs and irradiation. Nevertheless, cancer cells are often characterized by changes in gene encoding for proteins involved in this type of programmed cell death and survival signalling. Therefore the identifications of the genes and gene products that regulate apoptosis, together with an increased understanding about their mechanisms of acting, has laid the base for the finding of new drugs and new therapy methods to target apoptotic cell death [3,4,11,14]. Due to the new approaches in this area of interest, the aim of this research was to evaluate antioxidant and cytotoxic, pro-apoptotic potential of methanol extract of *C. majus* L. (CME) on two tumor cell lines and contribute to its potential effective employment in leukemia therapy.

MATERIAL AND METHODS

Preparation of extract from Chelidonium majus L. (CME)

The aerial parts of *C. majus* were collected, dried, cut into small pieces and extracted with warm methanol in the Soxhlet extractor (Sigma-Aldrich Co.) (4 cycles were run). The solvent was removed using a rotary evaporator (Rotary evaporator, BUCHI B-480, Switzerland) and after methanol removing, the solid green substance was extracted again in the Soxhlet extractor (4 cycles were run) with a warm hexane on purpose to remove chlorophyll. In addition, the extract was filtered and dried on air to yield the final extract powder. For the biological evaluation the extract was dissolved in DMSO and a 50 mg/mL solution was prepared.

DPPH radical scavenging assay

The antioxidant activity of CME was measured in terms of DPPH free radical scavenging ability, with slight modifications [15]. Ascorbic acid (5–500 μM) was used as reference compound. The ethanol solution of CME (50 μL) at different concentrations (100–1,000 μg/mL) was placed in a cuvette and 1 mL of 23.7 μg/mL ethanol solution of DPPH radical was added followed by incubation for 30 min. The decrease in absorbance at 517 nm was determined with a Genesys 10Bio spectrophotometer. All determinations were performed in three replicates.

Cell lines

Murine L1210 leukemia cells and human promyelocytic HL-60 leukemia cells were obtained from American Type Culture Collection (Rockville, MD, USA). Cells were grown in RPMI-1640 medium supplemented with 10% FCS, 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mM L-glutamine in an atmosphere of 5% CO_2 in humidified air at 37 °C. In all experiments exponentially growing cells were used.

Cytotoxicity assessment

Cytotoxicity was measured by the trypan blue assay and by the cell morphology monitoring. All experiments were performed in Petri dishes (Ø 60 mm). The cells were plated at a density of 2 x 10^5 cells/mL, treated with 10–100 μg/mL of CME and incubated for 24 h. The cell number and viability were determined by 0.4% trypan blue staining.

Apoptosis determination by fluorescence microscopy

Cells treated with 10–100 μg/mL CME for 24 h were resuspended in 1 mL of fresh medium, Hoechst 33342 (6 μg/mL) and propidium iodide (5 μg/mL) where added to the cell suspension and incubated for 30 min at 37 °C. Cells were centrifuged, resuspended in 40 μL of fresh medium and morphology was monitored by fluorescence microscopy (Olympus, Japan).

RESULTS

In the course of screening natural compounds for their potential application in treatment of cancer, we found out that the methanol extract of *C. majus* L. had the strong antioxidant activity; in the DPPH assay was able to scavenge DPPH in the dose-dependent manner (Figure 1).

The human promyelocytic leukemia HL-60 and murine leukemia L1210 cells were treated with CME in the concentration range from 10–100 µg/mL. CME extract acted cytotoxically in the dose-dependent manner towards both tumor cell lines (Figures 2 and 3). The human promyelocytic leukemia HL-60 cells were much more sensitive to CME treatment than the murine leukemia L1210 cells. The *in vitro* cytotoxic studies were complemented by the potential pro-apoptotic activity examination of CME assessed by fluorescence microscopy (Figure 4A and 4B). There was a considerable difference between negative control (Figure 4A) and leukemia cells treated with CME (Figure 4B), because of the ability of extract to induce apoptosis.

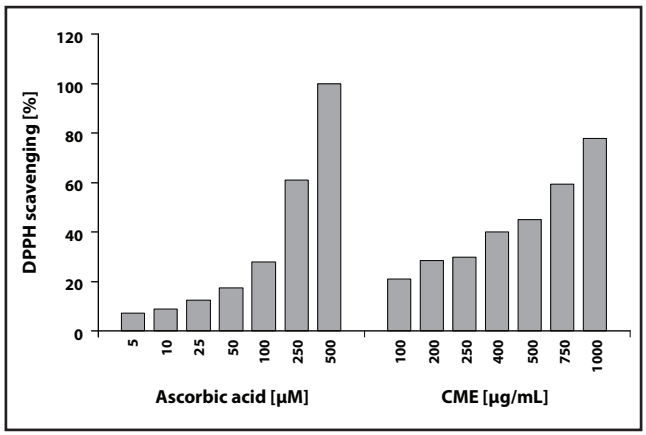

Figure 1. Antioxidant activity of CME determined by the DPPH radical scavenging assay. Values represent the data of three independent experiments (SD<5%). Ascorbic acid was used as a reference compound

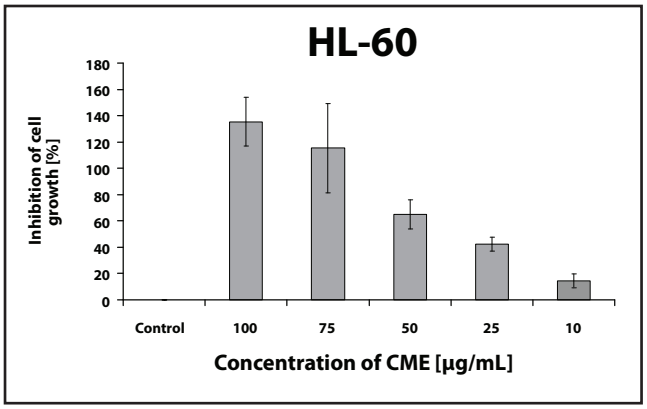

Figure 2. Cytotoxicity evaluation of CME in the human promyelocytic leukemia line HL-60 using the trypan blue staining. Cytotoxicity was decreased in the dose-dependent manner.

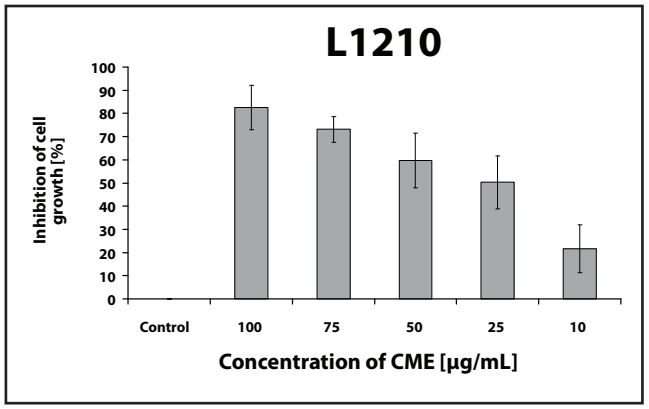

Figure 3. Cytotoxicity evaluation of CME in the murine leukemia line L1210 using the trypan blue staining. Cytotoxicity was decreased in the dose-dependent manner.

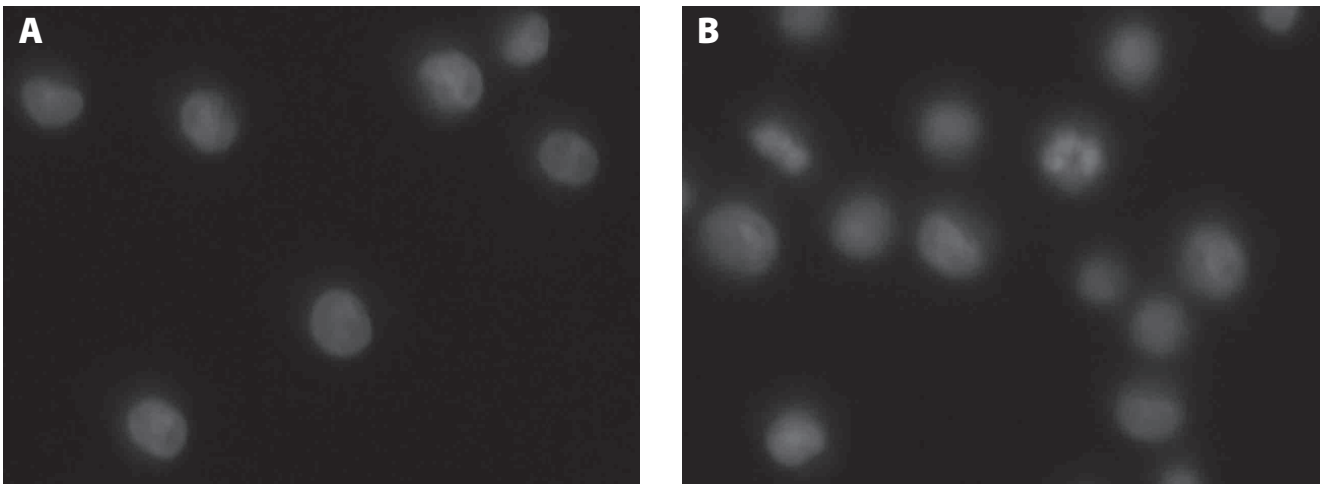

Figure 4. Fluorescence microscopy monitoring of apoptosis after 24 h in L1210 untreated control cells (**A**) and in L1210 cells treated with 50 µg/mL CME (**B**).

DISCUSSION

The antioxidant activity of CME revealed by the DPPH radical scavenging assay was probably mediated by its bioactive components – flavonoids, similarly as was formerly proved in our laboratory after antioxidant activity evaluation of extract isolated from artichoke *Cynara cardunculus* L. [10]. Then et al. [16] revealed that content of alkaloids and their seasonal oscillation in *C. majus* did not affect antioxidant property of extract investigated by FRAP method. The findings that the human promyelocytic leukemia HL-60 cells were more sensitive to CME treatment than the murine leukemia L1210 cells might be explained by differences in enzymatic equipments and genetic backgrounds of both leukemia cell lines used. This fact is promising regarding to its potential employment in an alternative human leukemia medication. Similarly as we revealed the apoptotic potential of CME on murine L1210 cancer cell line, apoptotic activity of milky sap isolated from *C. majus* L. was demonstrated also in human cervical carcinoma HeLa cell line by Nawrot et al. [12]. Based on results obtained it can be concluded that CME due to the presence of the isoquinoline alkaloids and the flavonoid components may play an important role in cancer chemoprevention and chemotherapy.

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