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In vitro cytotoxic and apoptotic activity of four Persian medicine plants on human leukemia and lymphoma cells

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PEER REVIEW

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Comments

This is a good study in which the authors evaluated the anti-tumor and cytotoxic activities on cancer cells *in vitro*. The results are interesting and suggested new potential chemotherapeutic agent for the treatment of lymphoma and leukemic cells.

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ABSTRACT

Objective: To investigate the cytotoxic and apoptotic activity of *Ferulago angulata*, *Echinophora platyloba*, *Salvia officinalis* and *Chelidonium majus* on leukemia and lymphoma cell lines, non-Hodgkin's B-cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), human acute myelocytic leukemia (KG-1A) cell lines and peripheral blood mononuclear cells.

Methods: Cytotoxicity was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cell viability assay was done using trypan blue exclusion experiments and cell death was identified as apoptosis using death detection ELISA.

Results: Our results demonstrated that the extracts dose and time dependently suppressed the proliferation of three leukemia and lymphoma tumor cell lines (KG-1A, U937 and Raji) with ascending order of IC₅₀ values, while peripheral blood mononuclear cells were not significantly affected. Nucleosome productions in apoptotic KG-1A, U937, and Raji cells were significantly augmented in a time-dependent manner and paralleled the anti-proliferative activity of the extracts.

Conclusions: The extracts were found to time- and dose-dependently inhibit the proliferation of KG-1A, U937, and Raji cells possibly via an apoptosis-dependent pathway.

KEYWORDS

Cytotoxicity, Apoptosis, Leukemia, Lymphoma, Herbal plant extract

1. Introduction

One of the conventional modality used in drug discovery, is herbal medicine as alternative cancer therapy due to their low toxicity or damage to normal cells. Extensive leukemia and lymphoma research evidences have confirmed cytotoxic effects of traditional medicinal plants

in inducing apoptosis on tumor cells[1]. New therapeutic agents include etoposide, camptothecin, VM26, vincristine, cis-platinum, cyclophosphamide, paclitaxel (taxol), 5-fluorouracil and doxorubicin isolated from medicinal plants, which show no toxic side effects, are vital in cancer treatment as current chemotherapeutic agents[2]. Most of these cancer chemo-preventive or therapeutic

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agents exert their chemotherapeutic activity by triggering apoptotic cell death^[3]. Therefore, induction of apoptosis in tumor cells has become an indicator of the tumor treatment response in employing a plant derived–bioactive substance to reduce and control human mortality due to leukemia and lymphoma^[4].

Apoptosis is associated with the rapid engulfment and removal of cell corpses by phagocytic cells. It is a vital component of several processes comprising normal cell turnover, proper development and functioning of the immune system, hormone–dependent atrophy, embryonic development and chemical–induced cell death^[5]. Inappropriate apoptosis is a factor in many human conditions including neuro–degenerative diseases, ischemic damage, autoimmune disorders and many types of cancer. The ability to modulate the life or death of a cell is recognized for its immense therapeutic potential^[6]. Studies revealed a high frequency of apoptosis in spontaneously regressing tumor and in tumor treated with cytotoxic anti–cancer agents. Traditionally, many extracts from roots, stems and fruits have been used in traditional medicine for maintaining health, enhancing overall immune status, and prevention and treatment of chronic diseases and the modulation and treatment of different diseases^[7,8]. Some of these substances are supposed to have potential value as cancer chemo–preventive and therapeutic agents within the human body^[9]. One of the approaches used in drug discovery is the ethno medical data approach, in which the selection of a plant is based on the prior information on the folk medicine use of the plant^[10].

Ferulago angulata (*F. angulata*), *Echinophora platyloba* (*E. platyloba*), *Salvia officinalis* (*S. officinalis*) and *Chelidonium majus* (*C. majus*) in Iranian traditional medicine exhibited a broad spectrum of biological activities and pharmacological properties, such as antioxidant, anti–microbial, anti–inflammatory, analgesic, hemostatic, and antitumor. Regulation of apoptosis is relevant for the development of hematologic malignancies like leukemia and lymphoma^[11–14]. In this study, the activity of four crude extract on apoptosis inducing effects was investigated on panel cancer cell line: non–Hodgkin’s B–cell lymphoma (Raji), human leukemic monocyte lymphoma (U937), Human acute myelocytic leukemia (KG–1A) cell lines and peripheral blood mononuclear cells (PBMCs). Cytotoxicity was determined by the 3–(4,5–dimethylthiazol–2–yl)–2, 5–diphenyltetrazolium bromide (MTT) assay. Cell viability assay using trypan blue exclusion experiments and cell death was identified as apoptosis using death detection ELISA.

2. Materials and methods

2.1. Preparation of extract

F. Angulata, *E. platyloba*, *S. officinalis* and *C. majus* plants were collected from the native habitats of Iran. The plants were identified by the Department of Botany, Institute of Medicinal Plants of Karaj, Iran. A voucher specimen was deposited in the herbarium of the above mentioned (No: 83IPM, 217IPM, 47IPM and 1459IPM respectively). The aerial parts of the plant were separated, shade dried and grinded into powder using mortar and pestle. The prepared

powder was kept in tight containers protected completely from light. Extraction of methanolic extract was carried out by macerating 100 g of powdered dry plant in 500 mL of 70% methanol for 48 h at room temperature. Then, the macerated plant material was extracted with 70% methanol solvent using percolator apparatus (two liter volume) at room temperature. The plant extract was removed from percolator, filtered through Whatman filter paper (No. 4) and dried under reduced pressure at 37 °C with rotator evaporator before being added to methanol as the solvent. The methanol extract was filtered and concentrated by a rotary evaporator and then evaporated to dryness. Briefly, the concentrated plant extracts were dissolved in dimethyl sulphoxide (DMSO) (SIGMA, USA) to get a stock solution of 10 mg/mL. The sub–stock solution of 0.2 mg/mL was prepared by diluting 20 µL of the stock solution into 980 µL serum–free culture medium, RPMI 1640 (The final concentration of DMSO in the experiment should not exceed 0.5). The stock and sub–stock solutions were both stored at 4 °C.

2.2. Cell culture and treatment

Raji, U937, KG–1A and human umbilical vein endothelial cell lines were purchased from National Cell Bank of Iran (Pasteur Institute, Tehran, Iran). The cells were grown in RPMI 1640 medium supplemented with 10% (v/v) fetal bovine serum (FBS), 100 IU/mL penicillin and 100 µg/mL streptomycin. Cells were incubated and maintained in a humidified incubator contain 5% CO₂ atmosphere at 37 °C (all prepared from Sigma, Germany). Upon reaching 80% confluence, the cells were passed into T75 culture flasks (Nunc, Denmark) and 10000 cells from log phase cultures were seeded in 100 µL of RPMI medium supplemented with 10% FBS per well of 96–well culture plates (Nunc, Denmark). Cells were incubated with the extracts for a defined time (12, 24 and 36 h). Blood samples from healthy volunteers were collected by venipuncture and transferred into 15 mL heparin coated test tubes. The blood was diluted with an equal amount of phosphate buffered saline (PBS) pH 7.4, and the PBMCs were separated by density gradient centrifugation (800 r/min for 20 min) over Ficoll–Hypaque density gradient at 20 °C. A total of 3×10⁶ PBMCs were washed and suspended in RPMI 1640 supplemented with 10% (v/v) FBS in 24–well plates and allowed to be settled for 2 h. Wells were then rinsed to remove non–adherent cells. Monocytes were maintained in RPMI 1640 supplemented with 10% (v/v) FBS at 37 °C, in 5% CO₂^[15]. Proliferative response and cell death of the extracts–treated cells were determined using MTT cytotoxicity assay, dye exclusion dye, and cell death detection ELISA, respectively. The experiments were performed in triplicate.

2.3. MTT cytotoxicity assay

The assay detects the reduction of MTT (a colorimetric technique) by mitochondrial dehydrogenase to blue formazan product, which reflects the normal function of mitochondria and hence for measuring the cytotoxicity cell and viability. A total of 1×10⁴ viable cells/well were plated into the 96–well tissue culture plates (Nunc, Denmark), and then incubated at 37 °C overnight. The next day when cells reached >80% confluence, the media were replaced with 200 mL of fresh complete medium containing 50, 100,

200, 300, 400, 500, 600, 800 µg/mL concentrations of crude extract, no extract with identical volume of solvent DMSO was added to the negative control well (final concentration of 0.5%). A chemotherapeutic anti-tumor drug, taxol at a final concentration of 20 µg/mL was added as the positive control. After 12, 24, or 48 h, the supernatants were removed and cell layers were washed with PBS (Invitrogen Gibco), and incubated with MTT (50 mL, 0.5 mg/mL) in RPMI 1640 without FBS for 4 h in a humidified atmosphere at 37 °C according to the manufacturer's protocol. The cell cultures were centrifuged at 500 r/min for 5 min and the supernatants were discarded. Subsequently, 200 µL of DMSO (Sigma) and 25 µL soreson buffer were added to dissolve the formazan crystals formed. The optical density (OD) colored solution was quantified at 570 nm wavelengths by an enzyme linked immunoabsorbent assay reader (ELISA Reader, Bio-Rad). The absorbance of untreated cells was considered as 100%. Each extract and control was assayed in triplicate in three independent experiments. Fifty percent of inhibition concentration (IC₅₀) was calculated by Excel software and the mean OD±standard deviation (SD) for each group of the replicates was calculated[16]. Percent growth inhibition of cells exposed to treatments was calculated as follows: % inhibition=100-(test OD/non-treated OD)×100. Concentration that inhibits 50% of cell growth was used as a parameter for cytotoxicity.

2.4. Dye exclusion assay

Cellular viability was measured using trypan blue dye exclusion assay. The cells were stained with trypan blue dye. Briefly, 1×10⁴ viable cells/well were seeded into 96-well tissue culture plates and then incubated at 37 °C overnight. The next day when cells reached >80% confluence, the media were replaced with 200 µL of fresh complete medium containing 0 [(0.5% (v/v) DMSO], 50, 100, 200, 300, 400, 500, 600, 800 µg/mL concentrations of crude extract, no extract was added to the negative control well for 12, 24 and 36 h. the cultures were harvested and wash twice with PBS. The cell pellet was then suspended with 0.5 mL PBS. Then, 20 µL of cell was mixed with equal volume of 0.4% trypan blue (Merck, Germany) and, the numbers of viable and non-viable cells were counted using Neubauer haemocytometer (Weber, England) under clear field microscopy (Nikon, Japan). The number of viable cells was calculated according to the following formula: [The unstained cell count (viable cells)×the dilution of the cell suspension×10⁴]/ the number of hemacytometer squares that were counted. The percent viability was calculated as: (viable cells/the total cell count) ×100. Each extract and control was assayed in triplicate in three independent experiments.

2.5. Cell death detection

Cell Death Detection ELISA PLUS kit (Roche Diagnostics GmbH, Germany) was used to measure apoptosis and necrosis, and then quantify histone-complexed DNA fragments (nucleosomes) in cytoplasm of the apoptotic cells after induction of apoptosis. The assay is based on a one-step sandwich ELISA principle as described elsewhere. Briefly, after incubation with the methanolic extract (near IC₅₀ concentration) and 0.5% (v/v) DMSO for 24 h cells were

pelleted and lysed. Mouse monoclonal antibodies against single-strand DNA and histones (H1, H2a, H2b, H3 and H4) were specifically detected and bound mononucleosomes and oligonucleosomes derived from cells undergoing apoptosis. The procedure was performed according to the manufacturer's protocol. Briefly, biotinylated anti-histone antibodies then fixed the antibody-nucleosome complexes to the streptavidin-coated microtiter plate. The anti-DNA antibodies were conjugated with a peroxidase that reacted with the substrate 2,2V-azino-di(3-ethylbenzthiazolin-sulfonate) to form a colored product. The remaining steps were carried out according to the instructions supplied by the manufacturer. The resulting color development, which was proportional to the amount of nucleosomes captured in the antibody sandwich, was measured at 405 nm wavelength by a benchmark microtiter plate reader (Bio-Rad)[17]. Results were expressed as the apoptotic index, calculated from the ratio of absorbance of treated (apoptotic) sample to that of the untreated (control) sample.

2.6. Statistical analysis

The data are expressed as mean±SD for at least three independent determinations in triplicate for each experimental point. The data were analyzed using IBM SPSS Statistics 20 software. For all the measurements, Two-way ANOVA followed by Duncan's new multiple range test ($P\leq 0.05$) was used to assess the statistical significance of difference between control and extract treated.

3. Results

The effect of *F. angulata*, *E. platyloba*, *S. officinalis* and *C. majus* on cell growth was measured in three hematological cell lines (Raji, KG-1A and U937). Cell growth was assessed indirectly by MTT assay. This assay gives an indication of undisturbed mitochondrial, also extra mitochondrial, NADH- and NADPH-dependent redox enzyme systems.

The effect of all extracts was studied as a dose and time response experiment after 12, 24, and 48 h at concentrations of 50 to 800 µg/mL with more than 90% suppression. All three cell lines exhibited significant suppression of growth above a concentration of 100 µg/mL but these extract exerted no significant growth inhibition on PBMC with IC₅₀ value greater than 800 µg/mL (Table 1–4).

Table 1

IC₅₀ values on inhibitory growth of four tumor cell lines by methanolic extract of *F. angulata*.

Extracts	Cell line	Time (h)	IC ₅₀ (µg/mL)±SD	
<i>F. angulata</i>	Raji	12	186.05±1.20	
		24	123.74±2.50	
		48	97.21±2.70	
	U937	12	297.51±1.20	
		24	205.36±2.10	
		48	158.00±1.00	
	KG-1A	KG-1A	12	202.71±3.50
			24	208.67±2.50
			48	182.44±4.30
PBMC		12	≥800	
		24	≥800	
		48	≥800	

Table 2

IC₅₀ values on inhibitory growth of four tumor cell lines by methanolic extract of *E. platyloba*.

Extracts	Cell line	Time (h)	IC ₅₀ (μg/mL)±SD
<i>E. platyloba</i>	Raji	12	201.89±3.40
		24	154.60±5.60
		48	125.94±1.70
	U937	12	279.51±1.10
		24	230.16±1.50
		48	178.42±0.70
	KG-1A	12	255.00±1.80
		24	167.19±2.90
		48	40.39±2.80
	PBMC	12	≥800
		24	≥800
		48	≥800

Table 3

IC₅₀ values on inhibitory growth of four tumor cell lines by methanolic extract of *S. officinalis*.

Extracts	Cell line	Time (h)	IC ₅₀ (μg/mL)±SD
<i>Salvia officinalis</i>	Raji	12	286.04±1.30
		24	196.62±0.80
		48	166.89±2.20
	U937	12	401.81±0.60
		24	319.16±1.30
		48	205.11±1.30
	KG-1A	12	402.69±0.90
		24	256.23±1.10
		48	179.00±3.20
	PBMC	12	≥800
		24	≥800
		48	≥800

Table 4

IC₅₀ values on inhibitory growth of four tumor cell lines by methanolic extract of *C. majus*.

Extracts	Cell line	Time (h)	IC ₅₀ (μg/mL)±SD
<i>C. majus</i>	Raji	12	191.27±1.20
		24	104.72±2.50
		48	73.74±1.10
	U937	12	252.81±2.10
		24	196.67±3.60
		48	138.66±2.40
	KG-1A	12	189.50±1.80
		24	113.65±4.10
		48	81.92±0.90
	PBMC	12	≥800
		24	≥800
		48	≥800

There were differences between leukemia cell lines in susceptibility towards growth inhibition by *S. officinalis* and *C. majus*. The IC₅₀ of *S. officinalis* in Raji cells was about two-fold lower than KG-1A, and U937 ($P<0.05$) and moreover growth inhibition of U937 was more resistant in comparison to Raji and KG-1A (Table 3 and 4). No significant difference in sensitivity towards *F. angulata*, *E. platyloba*, *S. officinalis*, and *C. majus* could be detected between Raji, KG-1A and U937 cells and they could significantly inhibit the proliferation of Raji, KG-1A, and U937 in a dose and time dependent manner.

To understand which the extract inhibits DNA synthesis in cells trypan blue exclusion experiments were performed. Following direct counting for non-viable and viable cells

using the trypan-blue exclusion test showed that 85% extract-treated with the highest concentration (400 μg/mL) did not absorb the dye at 24 h. Based on the results, extracts were further used to characterize the cell death mechanism.

Based on the IC₅₀ values ranging from 100 to 300 μg/mL as determined by MTT assay, 100, 200, and 300 μg/mL of the extract were chosen for cell death detection ELISA and incubated with each of the three cell lines (Raji, U937, and KG-1A) in 24 h. An increase in nucleosome production greater than or equal to two-fold was considered significant when compared with the untreated control. After 24 h, the extract at 100, 200, and 300 μg/mL significantly induced apoptosis of Raji, KG-1A and U937 cells in a dose-dependent manner (Table 5). No significant nucleosome production was found in PBMC after incubation with the extract at 24 h.

Table 5

The percentages of apoptosis and necrosis in three hematological cell lines (Raji, KG-1A and U937).

extracts	Concentration (μg/mL)	Cells	Apoptosis (%)	Necrosis (%)
FA	100	Raji	39.32±2.10	7.65±0.10
		KG-1A	59.45±0.90	8.00±2.90
		U937	45.33±1.10	7.67±2.10
		Raji	68.66±0.80	9.11±2.60
		KG-1A	59.53±1.20	9.00±1.70
		U937	54.00±0.00	6.13±0.80
	200	Raji	70.81±0.00	11.00±2.10
		KG-1A	61.61±0.20	10.34±1.20
		U937	56.65±1.60	6.50±0.90
		Raji	48.63±1.20	7.60±0.40
		KG-1A	43.40±3.10	7.93±0.60
		U937	52.23±2.00	7.11±1.10
300	Raji	54.00±2.10	9.00±20.00	
	KG-1A	45.32±3.20	9.00±0.50	
	U937	50.68±2.80	8.68±2.20	
	Raji	57.12±1.60	13.10±1.50	
	KG-1A	47.72±2.20	11.00±0.50	
	U937	52.46±1.60	9.10±1.30	
SO	100	Raji	56.00±4.90	8.69±0.40
		KG-1A	56.72±0.50	7.69±5.40
		U937	55.65±0.40	7.00±0.60
		Raji	60.67±1.10	9.45±3.30
		KG-1A	62.43±6.10	9.34±0.20
		U937	59.32±1.90	8.65±2.70
	200	Raji	66.64±2.60	10.91±1.50
		KG-1A	65.64±1.00	10.66±7.80
		U937	61.89±2.40	9.13±2.00
		Raji	44.53±1.10	60.71±0.700
		KG-1A	41.55±1.90	47.34±0.4
		U937	42.39±0.40	43.35±5.50
300	Raji	47.00±1.80	65.00±0.60	
	KG-1A	43.45±1.40	50.35±2.80	
	U937	45.00±4.20	69.32±2.90	
	Raji	51.23±1.70	71.29±1.20	
	KG-1A	46.23±2.10	56.66±2.70	
	U937	47.34±0.50	73.15±4.70	

FA: *F. angulata*; EP: *E. platyloba*; SO: *Salvia officinalis*; CM: *C. majus*.

4. Discussion

Current treatment of malignant leukemia and lymphoma

typically includes radiation and chemotherapy; however, these modalities can damage other non-malignant cell and cause morbidity[6]. The identification of a novel alternative therapy that have tumor selective property without or less side effect on normal cells could substantially impact the morbidity and survival of patients with this disease[18].

Three leukemia and lymphoma cell lines (Raji, KG-1A and U937) and a normal human cell type (PBMC) were used for the cytotoxicity test. The cancerous cell lines possess differences in their origin, morphology and genomes, resulting in susceptibility differences to the chemotherapeutic agents. The *F. angulata*, *E. platyloba*, *S. officinalis* and *C. majus* extract in its natural form, significantly suppressed the proliferation of lymphoma and leukemia cells (Raji, KG-1A and U937) in tumor-selective, dose and time dependent manner *in vitro*, except PBMC cells. The highest cytotoxicity activity is given in the order: *C. majus* > *F. angulata* > *E. platyloba* > *S. officinalis*, respectively. In comparison, to the crude extract of *C. majus* exhibited highest significant cytotoxic activity against all tumor cell lines with lower IC₅₀ percent values. Moreover, *F. angulata*, *E. platyloba* and *S. officinalis* showed a degree cytotoxic activity dependent on the cell line type.

Apoptosis is known as an important type of cell death in response to cytotoxic treatment. The administration of many natural compounds with anti-cancer effect has been shown to be capable of inducing the apoptotic death of cancer cells[9]. Our results illustrated that the extracts dose and time dependently induced apoptosis in Raji, KG-1A and U937 cells in terms of nucleosome production. These suggested that the extract exerted its anti-tumor effect on the three leukemia and lymphoma cells possibly via an apoptotic mechanism.

To our knowledge, there was no previous detailed mechanistic study on apoptosis induction of these extract on leukemia and lymphoma cells. The present findings showed that maximal increase in nucleosome production detected by cell death ELISA was observed earlier than MTT-based cytotoxicity in the three leukemia and lymphoma cell lines. In addition, the results of induced apoptosis were found to be paralleled with those of MTT assays in a descending order (Raji > KG-1A > U937).

Ampasavate *et al.* examined the anti-leukemic activity of non-edible parts of 13 common Thai tropical fruits[19].

Their ethanolic extracts were tested for cytotoxic effects on U937, K562, HL60, Molt4 and normal human PBMCs. Three of 20 crude plant extracts (kaffir lime leaves, mangosteen peels, and wampee leaves) had strong cytotoxic effects on K562, U937, and Molt4 cells. The IC₅₀ values of kaffir lime leaves on those cells were 26.1, 9.0 and 11.9 µg/mL respectively, whereas those of mangosteen peel were 23.6, 4.5 and 10.1 µg/mL, and those of wampee leaves were 71.9, 13 and 70.4 µg/mL. Furthermore, pomegranate peel extract had a potent cytotoxic effect on HL60 cells (IC₅₀ of 8.0 µg/mL), but was non-toxic to normal PBMCs, indicating that

it can be viewed as a potential source of anti-leukemic agents[19].

Muller *et al.* studied anti-proliferative activity on *Ganoderma lucidum* (*G. lucidum*) extract against 26 human cancer cell lines[20]. The six most sensitive hematologic cell lines were: HL-60 (ED₅₀ 26 µg/mL), U937 (63 µg/mL), K562 (50 µg/mL), Blin-1 (38 µg/mL), Nalm-6 (30 µg/mL) and RPMI 8226 (40 µg/mL). Cell cycle analyses revealed a G2/M arrest, most prominently in HL60 cells. Four hematopoietic cell lines (HL60, Blin-1, U937, and RPMI 8226) were examined for apoptosis, which ranged between 21% and 92%. After exposure to *G. lucidum* extract, HL60 cells became multinucleated with an increased DNA content. These results indicate that *G. lucidum* extract has a profound activity against leukemia, lymphoma and multiple myeloma cells may be a novel adjunctive therapy for the treatment of hematologic malignancies[20].

In order to elucidate the cytotoxic activities of extracts on the growth of leukemia and lymphoma cells of other sub-types, acute T-cell leukemia cell line (Jurkat) and Hodgkin's lymphoma may become the target cells used in our future studies. In addition, mechanistic studies on cell cycle arrest and early apoptotic events may be conducted to delineate other possible anti-tumor mechanisms of the extracts. Besides, future *in vivo* anti-tumor studies will be performed in order to confirm these *in vitro* results.

This study provides the evidence that *in vitro* cytotoxic activity of methanolic *C. majus*, *F. angulata*, *E. platyloba* and *S. officinalis* extracts were found to tumor-selectively and dose and time dependently inhibit the proliferation of lymphoma and leukemic cells possibly via an apoptosis-dependent pathway.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

F. angulata, *E. platyloba*, *S. officinalis* and *C. majus* have been used in traditional medicine that believed to have cytotoxic properties, although the mechanism for the activity remains to be elucidated. Cytotoxicity was determined by the MTT assay. Cell viability assay is done

using trypan blue exclusion experiments and cell death was identified as apoptosis using Death Detection ELISA.

Research frontiers

In this study, the cytotoxic and apoptotic activity of these plants was investigated on leukemia and lymphoma cell lines, Raji, U937, KG-1A cell lines and PBMCs.

Related reports

This study provides the evidence that *in vitro* cytotoxic activity of methanolic *C. majus*, *F. angulata*, *E. platyloba*, and *S. officinalis* extracts were found to tumor-selectively and dose and time dependently inhibit the proliferation of lymphoma and leukemic cells possibly via an apoptosis-dependent pathway. Data of the present study is somehow similar to the findings of other researchers that the *E. platyloba* extract with time and dose dependently inhibit the proliferation of fibrosarcoma cell possibly via an apoptosis-dependent pathway.

Innovations & breakthroughs

The identification of a novel alternative therapy that have tumor selective property without or less side effect on normal cells could substantially impact the morbidity and survival of patients with this disease. The present study demonstrated the *in vitro* cytotoxic and apoptotic activity of these plants lymphoma and leukemic cells, possibly suggesting a new potential chemotherapeutic agent for the treatment of lymphoma and leukemic cells.

Applications

The present study suggests a new potential chemotherapeutic agent for the treatment of lymphoma and leukemic cells.

Peer review

This is a good study in which the authors evaluated the anti-tumor and cytotoxic activities on cancer cells *in vitro*. The results are interesting and suggested new potential chemotherapeutic agent for the treatment of lymphoma and leukemic cells.

References

- [1] Mehta RG, Murillo G, Naithani R, Peng X. Cancer chemoprevention by natural products: how far have we come? *Pharm Res* 2010; **27**(6): 950–961.
- [2] Fulda S. Evasion of apoptosis as a cellular stress response in cancer. *Int J Cell Biol* 2010; doi: 10.1155/2010/370835.
- [3] Abou-Nassar K, Brown JR. Novel agents for the treatment of chronic lymphocytic leukaemia. *Clin Adv Haematol Oncol* 2010; **8**(12): 886–895.
- [4] Small S, Keerthivasan G, Huang Z, Gurbuxani S, Crispino JD. Overexpression of survivin initiates haematologic malignancies *in vivo*. *Leukaemia* 2010; **24**(11): 1920–1926.
- [5] de Bruin EC, Medema JP. Apoptosis and non-apoptotic deaths in cancer development and treatment response. *Cancer Treat Rev* 2008; **34**: 737–749.
- [6] Whelan RS, Kaplinskiy V, Kitsis RN. Cell death in the pathogenesis of heart disease: mechanisms and significance. *Annu Rev Physiol* 2010; **72**: 19–44.
- [7] O'Brien MA, Kirby R. Apoptosis: a review of pro-apoptotic and antiapoptotic pathways and dysregulation in disease. *J Vet Emerg Crit Care* 2008; **18**(6): 572–585.
- [8] Wylie AH. "Where, O death, is thy sting?" a brief review of apoptosis biology. *Mol Neurobiol* 2010; **42**(1): 4–9.
- [9] Fulda S, Galluzzi L, Kroemer G. Targeting mitochondria for cancer therapy. *Nat Rev Drug Discov* 2010; **9**: 447–464.
- [10] Moon JY, Mosaddik A, Kim H, Cho M, Choi HK, Kim YS, et al. The chloroform fraction of guava (*Psidium cattleianum* Sabine) leaf extract inhibits human gastric cancer cell proliferation via induction of apoptosis. *Food Chem* 2011; **125**: 369–375.
- [11] Lima CF, Andrade PB, Seabra RM, Fernandes-Ferreira M, Pereira-Wilson C. The drinking of a *Salvia officinalis* infusion improves liver antioxidant status in mice and rats. *J Ethnopharmacol* 2005; **97**(2): 383–389.
- [12] Rechinger KH. *Flora Iranica*. Graz, Austria: Akademische Druck und Verlagsanstalt; 1987, p. 72.
- [13] Gilca M, Gaman L, Panait E, Stoian I, Atanasiu V. *Chelidonium majus*, an integrative review: traditional knowledge versus modern findings. *Forsch Komplementmed* 2010; **17**(5): 241–248.
- [14] Mazloomifar H, Saber-Tehrani M, Rustaiyan A. Constituents of the essential oil of *Echinophora platyloba* DC growing wild in Iran. *J Essent Oil Res* 2004; **16**(4): 284–285.
- [15] Shahneh FZ, Baradaran B, Orang M, Zamani F. *In vitro* cytotoxic activity of four plants used in Persian traditional medicine. *Adv Pharm Bull* 2013; **3**(2): 453–455.
- [16] Shahneh FZ, Valiyari S, Azadmehr A, Hajiaghvae R, Yaripour S, Bandehagh A, et al. Inhibition of growth and induction of apoptosis in fibrosarcoma cell lines by *Echinophora platyloba* DC: *in vitro* analysis. *Adv Pharmacol Sci* 2013; doi: 10.1155/2013/512931.
- [17] Valiyari S, Baradaran B, Delazar A, Pasharan A, Zare F. Dichloromethane and methanol extracts of scrophularia oxysepala induces apoptosis in MCF-7 human breast cancer cells. *Adv Pharm Bull* 2012; **2**(2): 223–231.
- [18] Lau CB, Ho CY, Kim CF, Leung KN, Fung KP, Tse TF, et al. Cytotoxic activities of *Coriulus versicolor* (Yunzhi) extract on human leukemia and lymphoma cells by induction of apoptosis. *Life Sci* 2004; **75**: 797–808.
- [19] Ampasavate C, Okonogi S, Anuchapreeda S. Cytotoxicity of extracts from fruit plants against leukemic cell lines. *Afr J Pharm Pharmacol* 2010; **4**(1): 13–21.
- [20] Muller CI, Kumagai T, O'Kelly J, Seeram NP, Heber D, Koeffler HP. *Ganoderma lucidum* causes apoptosis in leukemia, lymphoma and multiple myeloma cells. *Leuk Res* 2006; **30**: 841–848.