J HerbMed Pharmacol. 2015; 4(2): 49-52.

Journal of HerbMed Pharmacology

Journal homepage: http://www.herbmedpharmacol.com

Cytotoxic activities of *Euphorbia kopetdaghi* against OVCAR-3 and EJ-138 cell lines

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ARTICLE INFO

Article Type:
Original Article

Article History:
Received: 19 November 2014
Accepted: 24 January 2015

Keywords:
Euphorbiaceae
Euphorbia kopetdaghi
cytotoxicity
OVCAR-3
EJ-138

ABSTRACT

Introduction: Over the centuries, the genus Euphorbia was known to be toxic to humans and animals. Recently, in a primary study significant suppressive activity against phytohemagglutinin activated T-cell proliferation has been reported from this plant. Therefore, this study was designed to evaluate the cytotoxic effects of different parts of *E. kopetdaghi* against cancer cell lines.
Methods: Filtration and in vacuo concentration resulted in a green gum which was subjected on silica gel CC (hexane/Acetone, 0→50) to several fractions: F1-F8. The inhibitory effects of obtained fractions with 5, 50, and 500 µg/ml concentrations were evaluated on proliferation and viability of cancer cells (OVCAR and EJ-138) in 48 hours treatment. Finally, cell viability was determined at a wavelength of 570 by 3-4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method.
Results: Based on studies of microscopic observation and viability testing, F1, F2, F4, F5, F6, and F7 showed significant cytotoxic effect at concentration of 50 and 500 µg/ml against EJ-138 and OVCAR-3 cell lines. These fractions inhibited growth of EJ-138 and OVCAR-3 cells in a concentration-dependent manner. Fraction of F8 induced tumor promotion significantly in EJ-138 and OVCAR-3 cells, respectively.
Conclusion: Due to the inhibitory properties of *E. kopetdaghi* extract and its fractions on cancer cells of OVCAR3 and EJ-13, isolation, purification and identification of compounds presented in the fractions possessing cytotoxic effects are recommended which were the area of our future research.

Implication for health policy/practice/research/medical education:
In the present study, we evaluated the cytotoxicity properties of E. kopetdaghi extract and its fractions on cancer cells of OVCAR3 and EJ-138. The observed moderated toxicity of fractions indicated that this plant has the value of more phytochemical consideration to find possible anti-cancer lead compounds.
Please cite this paper as: Aghaei M, Ghanadian M, Faez F, Esfandiary E. Cytotoxic activities of *Euphorbia kopetdaghi* against OVCAR-3 and EJ-138 cell lines. J HerbMed Pharmacol 2015; 4(2): 49-52.

Introduction

Throughout history, humans have used plant extracts for production of bio-active secondary metabolites required in their medications. Over the centuries, the genus Euphorbia has been known to be toxic to humans and animals. In traditional Iranian and Chinese medicine Euphorbia is used as anti-tumor and for elimination of warts (1,2). Recently, different species of Euphorbia showed cytotoxic effects against some cancer cell lines including antitumor properties of *Euphorbia prolifera* on ovarian cancer (3,4), *E. helioscopia* on breast cancer lines (5), and *E. guyoniana* against kidney cell line HEK293 (6). The genus *Euphorbia has also* a high diversity in Iran and represents 97 species in the *Flora Iranica* areas from which 56 with 17 endemics are growing in Iran and *E. kopetdaghi* is one of them (7). *E. kopetdaghi* is restricted in the kopedagh (northeastern Iran, northwestern Afghanistan and south Turkmenistan (7). Recently, in a primary study by Ghanadian *et al.* on this plant, significant suppressive activity against PHA activated T-cell proliferation and oxidative burst

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suppressive activity was reported (8). Therefore, this study was designed to evaluate the cytotoxic effects of different parts of *E. kopetdaghi* against two different cancer cell lines: Human ovarian carcinoma cancer cell line (OVCAR-3), and Human bladder carcinoma cancer cell line (EJ138).

Material and Methods

Plant material

The *Euphorbia kopetdaghi* was collected from Mashhad (Khorasan province, North East of Iran), in July 2013. The plant was authenticated by the Department of Forestry, University of Isfahan, Iran. The plant materials were stored at -20°C before use. A Voucher specimen no: 2024 of the plant was deposited in the herbarium unit of the School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

Extraction and isolation

Aerial parts of plant was extracted by ethylacetate. The extract was concentrated under reduced pressure at 40–45°C by rotatory vacuum evaporator (Heidolph, Germany) and resulted in a green gum, which was charged over a cake of 15% paraffin impregnated silica-gel (400 g) packed into a sintered Buchner funnel (150×90 mm) using MeOH: H2O (65:35) as eluent to remove fats and chlorophyl. The ungreased fraction lacked of its apolar constituents, was separated on silica gel CC (hexane/acetone, 0→50).

MTT viability assay

Human ovarian carcinoma cancer cell line OVCAR-3, and Human bladder carcinoma cancer cell line EJ138 were obtained from Pasteur Institute of Iran. The cell lines were grown in RPMI-1640 media supplemented with 10% fetal calf serum, 100 U/ml + 100 µg/ml penicillin and streptomycin at 37°C in 5% CO_2 condition. Cell viability was determined colorimetric assay, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and compared with untreated controls (9). Cells were seeded at 5×10^3 cells/well in 96-well plates. After incubation, compounds at concentrations of 5, 50, and 500 µg/ml were added and incubated again for 48 hours. Then, MTT (5 mg/ml in PBS) was added to each well and the cells were incubated for 4 hours. The supernatants were aspirated and 200 µl of dimethyl sulfoxide was added to each well. The plates were shaken for 10 minutes and using colorimetric method, the absorbance was read by the microplate reader (Bio-Rad, Hercules, CA, USA) at 570 nm. Cell viability was calculated based on the formula: (mean OD of treated cells/mean OD of control cells) × 100. The results expressed as percent of untreated cells. The criteria used to categorize the cytotoxicity of isolated fractions against cancer cell lines, based on U.S. National Cancer Institute (NCI) and Geran protocol (10,11) was as follows: $IC_{50} \leq 20$ µg/ml = highly cytotoxic, IC_{50} 21-200 µg/ml = moderately cytotoxic, IC_{50} 201-500 µg/ml = weakly cytotoxic and $IC_{50} > 501$ µg/ml = no cytotoxic.

Statistical analysis

One-way analysis of variance (ANOVA) was performed using Dennett's test as post hoc analysis. Each experiment was carried out in triplicate independently. $P<0.05$ was considered significant. All data are expressed as means ± SD.

Results

The isolated fractions of column chromatography were concentrated by a rotary evaporator at 40° C and combined based on their TLC profile to afford 8 combined fractions which are displayed in Table 1. Fr 1-8 stored in refrigerator at -20°C before use.

To investigate the cytotoxic effects of different factions of the *E. kopetdaghi*, on the cell viability, EJ-138 and OVCAR cell lines were incubated with the concentrations of 5, 50, and 500 µg/mL of fractions 1-8. After, 48 hours, cell cytotoxicity was evaluated using standard MTT assay.

Fraction F2 showed significant cytotoxic effect ($P<0.05$) at lower concentration of 5, 50 and µg/ml against EJ-138 cell line. F1, F4, F5, F6, and F7 showed significant cytotoxic effect at concentrations of 50 and 500 µg/ml against EJ-138 and OVCAR-3 cell lines. These fractions inhibited growth of EJ-138 and OVCAR-3 cells in a concentration-dependent manner.

Fraction F3 reduced tumor promotion significantly ($P<0.05$) at concentrations of 50 µg/ml but then slightly induced cell proliferation of viable cells at higher concentration of 500 µg/ml in both EJ-138 and OVCAR-3 cells. Fraction F8 induced tumor promotion, significantly ($P<0.05$) by 288.5%± 34.2, and 134.9%± 15.4 in EJ-138 and OVCAR-3 cells, respectively. Cytotoxicity activities of Fr 1-8 against cancer cell lines: EJ-138 and OVCAR-3 cells are displayed in Figure 1.

The IC_{50} was calculated for all of the fractions with cytotoxic activities (Table 2). Based on NCI and Geran protocol, Fractions F1, F2, F4, F5, F6 and F7 showed moderate cytotoxicity against OVCAR-3 and EJ-138 cancer cell lines.

Discussion

Since triterpenoids along with macrocyclic diterpenoids are the main phytochemicals isolated from the genus

Table 1. The solvent system employed for column chromatography

Combined Fraction	n-hexane: acetone
Fr. 1	84:16
Fr. 2	80:20
Fr. 3	75:25
Fr. 4	75:25
Fr. 5	70:30
Fr. 6	70:30
Fr. 7	65:35
Fr. 8	65:35

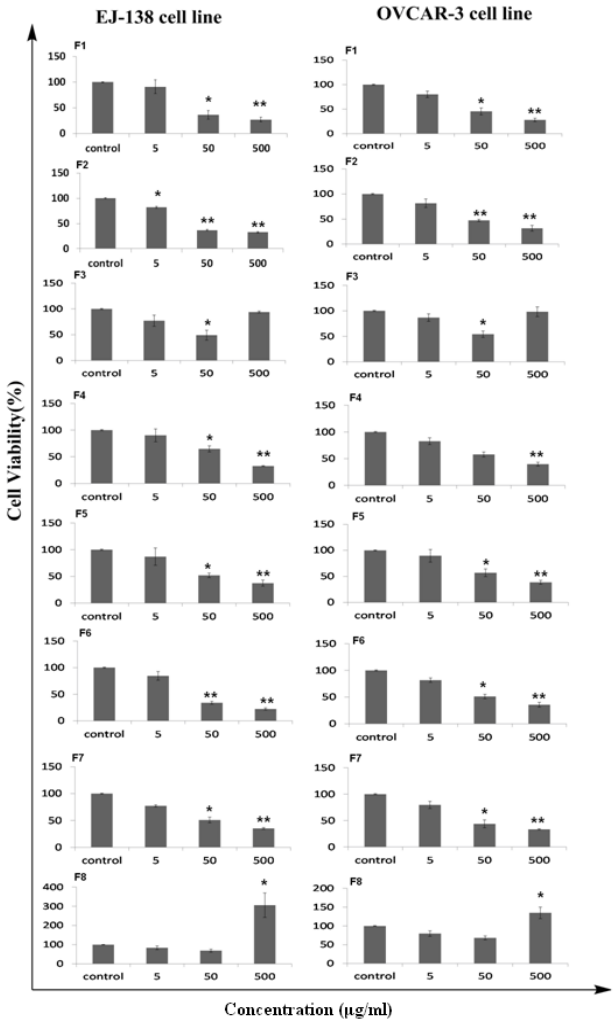

Figure 1. Cytotoxicity effect of different fractions: F1-F8 of *Euphorbia kopetdaghi* against ovarian (OVCAR-3) and bladder (EJ-138) cancer cell lines. Cells were treated with different concentrations of fractions 1-8 for 48 hours, and cytotoxicity was assessed by MTT assay. Fractions 1-8 reduced cell viability in OVCAR-3 and EJ-138 cell lines in a dose dependent manner. Results (mean ± SD) were calculated as percent of corresponding control values. *P<0.05, **P<0.001 are significant. Statistical analysis was performed by ANOVA.

Euphorbia (2), the observed cytotoxicity are mainly due to the presence of these compounds. None of the fractions showed high cytotoxic activity (IC_{50} < 20 µg/ml).

Inspected by NMR, Fr. 1 and Fr. 2 with typical signals of triterpenoids showed more cytotoxic effects. Moderate cytotoxic activities observed by Fr. 4-Fr. 7 are probably due to the presence of diterpenoid polyesters reported in the genus Euphorbia. The induced cell proliferation of viable cells by Fr. 8 at higher concentrations in both EJ-138 and OVCAR-3 cells may be due to the presence of phorbol or ingenol esters which are known for their cancer promoting effects.

Compared with literature, chloroform and ethyl acetate fractions of *Euphorbia wallichi*, as reported by Irshad Ali and co-workers, showed moderate cytotoxic activity at a concentration of 100 µg/mL (12). In a study conducted by Ping *et al.* at 2013, the extract of *E. hirta* showed significant toxicity against brine shrimp with an LC_{50} value of 620.382 µg/mL (13). On the other hand protective properties are also reported from *Euphorbia hirta* against antitubercular drug-induced cytotoxicity in freshly isolated hepatocytes (14). So, these results indicate that moderate cytotoxicity of the isolated fractions has the value of more phytochemical consideration and isolations of pure compounds responsible of the observed activities.

Table 2. IC_{50} values of *Euphorbia kopetdaghi* fractions against ovarian (OVCAR-3) and bladder (EJ-138) cancer cell lines

Fraction	Cytotoxic activity	EJ-138	OVCAR-3
		IC_{50} ± SD (µg/mL)	IC_{50} ± SD (µg/mL)
F1	Moderate	55.3 ± 6.6	62.3 ± 5.6
F2	Moderate	54.3 ± 2.3	70.2 ± 4.9
F3	No	>500	>500
F4	Moderate	132.4 ± 11.5	148.7 ± 12.3
F5	Moderate	115.7 ± 12.7	158.4 ± 17.4
F6	Moderate	38.9 ± 3.3	85.1 ± 7.1
F7	Moderate	79.6 ± 8.2	81.9 ± 6.8
F8	No	>500	>500

A fitted dose-response curve were plotted to enable the calculation of the concentrations that kills 50% of the cells (IC_{50}). The criteria used were as follows: IC_{50}<20 µg/ml (high cytotoxic activity), IC_{50}: 20-100 µg/ml (moderate cytotoxic activity), IC_{50}: 201-500 µg/ml (weak cytotoxic activity), IC_{50}>500 µg/ml (no cytotoxic activity).

Acknowledgments

This paper is part of the thesis of Farough Faez submitted for the fulfillment of the degree of Pharm D in Faculty of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Tehran, Iran.

Authors' contributions

MA co-ordinated the study and participated in most of the experiments. MG carried out the design and contributed in data analysis and writing and finalizing the manuscript. FF participated in most of the experiments and in manuscript preparation.

Conflict of interests

The authors have no conflict of interests.

Funding/Support

It has been supported by Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences (Grant No: 393059).

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