Iranian Journal of Basic Medical Sciences
Vol. 11, No. 4, Winter 2009, 250-253
Received: Nov 22, 2008; Accepted: Dec 26, 2008

IJBMS

Short communication

In Vitro Cytotoxicity of Two Subspecies of *Juniperus excelsa* on Cancer Cells

*[1]Hojjat Sadeghi-aliabadi, [2]Ahmad Emami, [1]Babak Sadeghi, [3]Abbas Jafarian

Abstract

Objective(s)
The cytotoxic effects of crude ethanol extracts of some previously tested Iranian conifers on tumor cell lines have motivated us to screen different parts of two subspecies in these genus.
Materials and Methods
Terminal branchlets and berries of *Juniperus excelsa* subsp. *excelsa* and *J. excelsa* subsp. *polycarpos* were collected, dried and extracted with ethanol/H_2O (80/20 v/v) via percolation procedure. Extracts were dried, reconstituted in ethanol and cytotoxic effects of different concentrations were determined on cancer cells by ELISA, using MTT assay. MDA-MB-468, Hela and KB cells were used in this study.
Results
The extracts of the branchlets of male and female of *J. excelsa* subsp. *polycarpos* as well as berries extract of *J. excelsa* subsp. *excelsa* showed inhibitory activities against KB cells. Extracts of female branchlets and berries of *J. excelsa* subsp. *polycarpos* were cytotoxic against all 3 cell lines.
Conclusion
In conclusion, obtained extracts from *J. excelsa* subsp. *polycarpos* showed cytotoxic effects against most tested cell lines which was comparable to doxorubicin; whereas, berries extracts of *J. excelsa* subsp. *excelsa* showed inhibitory effects only against KB cells.

Keywords: Cytotoxicity assay, Hela cells, *Juniperus excelsa*, *J. polycarpos*

1- Department of Pharmaceutical Chemistry, School of Pharmacy and Pharmaceutical Sciences and Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
* Corresponding author: Tel: +98-311- 7922564; Fax: +98-311-6680011; email: sadeghi@pharm.mui.ac.ir
2- Department of Pharmacognosy, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
3- Isfahan Pharmaceutical Sciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Introduction

Conifers are a small group of the flora of Iran (8 species from 8000 species). All aromatic Iranian conifers belong to Cupressaceae family. In Iran this family consists of one species of *Platycladus*, one species of *Cupressus* and five species of *Juniperus*.

Juniperus L. is the second most diverse genus of the conifers. The genus *Juniperus* consist of approximately 67 species and 28 varieties. The genus is divided into three sections: *Caryocedrus* Edlicher (with only one species); *Juniperus* (syn: *Oxycedrus* Spach with 12 species) and *Sabina* Miller Spach (with 55 species) (1). Two examined subspicies: *J. excelsa* subsp. *excelsa* and *J. excelsa* subsp. *polycarpos* in our study belong to latter section.

J. excelsa subsp. *excelsa*, with the Persian name of "Arduj" (2), is a medicinal plant and traditionally used for dysmenorrhea (3), cough (4), bronchitis and common cold, jaundice and tuberculosis (5) and to induce menses and induction of abortion. *J. excelsa* subsp. *polycarpos*, called "Ors" (2) in Persian, is used for asthma (6).

Our previous studies revealed that different parts of Iranian conifers possess cytotoxic effects on some tumor cell lines (7-9). It is believed that some lignans such as podophyllotoxin and desoxypodophyllotoxin are responsible for this effect. In the present study we sought to evaluate the cytotoxic effects of different parts of *J. excelsa* subsp. *excelsa* and *J. excels* subsp. *polycarpos* on Hela, KB and MDA-MB-468 cell lines.

Materials and Methods

Plant material

Male and female branchlet and berries of *J. excelsa* subsp. *polycarpos* and *J. excelsa* subsp. *excelsa* were collected from Aliabad Katol (2000 m, Golestan province, north of Iran) and Vinaq in Arasbaran (1950 m, East Azarbayejan province, north west of Iran) in September 2001, respectively. The plants were identified by the Department of Forestry, University of Tehran, Iran. The plant materials were stored at –20 °C before use. Voucher specimens of the plants (No. 1416-1417) were deposited in the Herbarium of School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, Iran.

Extraction and isolation

Fifty g of each plant (air-dried in 20 days at room temperature) was crushed and soaked in 75 ml of ethanol (80% v/v) for 24 hr and then percolated (5 hr, 30 drops/min) (10). The extracts were concentrated by a rotary evaporator and dried in an oven at 40 °C to give 0.5-0.8 g of solid residue. Twenty mg of solid residues were dissolved in one ml of ethanol and diluted to 100 ml with distilled water and filtered through 0.22 μm microbiological filters. Dilution was continued so that the final concentrations of extracts were 5, 10 and 20 μg/ml.

Cell lines

Hela, KB, and MDA-MB-468 cell lines were purchased from Pasture Institute, Tehran, Iran and grown in completed RPMI-1640.

MTT-based cytotoxicity assay

The cytotoxic effect of obtained extracts against cell lines was determined by MTT assay (11). Assay procedure and cell survival calculations were performed according to our previous study (7).

Statistical analysis

SIGMASTAT™ (Jandel Software, San Raphael, CA) was used to perform statistical analysis. Analyze-of-variance (ANOVA) followed by Dunkan test (as the post-hoc) was used to evaluate the differences between groups. Significance was set at the 5% level.

Results

Cytotoxic effect of extracts against Hela cells

Hydroalcoholic extracts of the terminal branchlets and berries of female *J. excelsa* subsp. *polycarpos* at 20 μg/ml, showed an inhibitory effect against Hela cells (Table 1). Although hydroalcoholic extracts of *J. excelsa* subsp. *excelsa* significantly decreased percentage of cell survival, IC_{50} was not obtained.

Cytotoxic effect of extracts against KB cells

Hydroalcoholic extracts of the branchlets and berries of male and female trees of *J. excelsa* subsp. *polycarpos* (5, 10 and 20 μg/ml) showed an excellent inhibitory effects against KB cells (IC_{50}<5 μg/ml; Table 1). For *J. excelsa* subsp. *excelsa* only the hydroalcoholic extracts of berries were cytotoxic against this cell line (IC_{50}<5 μg/ml; Table 1).

Table 1. Cytotoxic effects of hydro-alcoholic extracts of different parts of 2 subspecies of *Juniperus excelsa* against 3 cancer cell lines following 72 hr continuous exposure to each extract.

Cell lines	Concentration (µg/ml)	Plants					Controls	
		J. excelsa subsp. *polycarpos*			*J. excelsa* subsp. *excelsa*		Positive[§]	Negative[Φ]
		Branchlets (male)	Branchlets (female)	Berries	Branchlets	Berries		
Hela	5	87±7 *	80±8 *	105±19	88±18	100±6		
	10	70±12 *	69±2 *	63±3 *	87±19	66±4 *	30±6 *	100±8
	20	58±3 *	43±16 *	46±16 *	88±3 *	50±1 *		
KB	5	28±5 *	32±1 *	28±1 *	88±6 *	36±1 *		
	10	22±3 *	18±3 *	20± 6 *	75±14 *	29±5 *	23±2 *	100±6
	20	7±2 *	10±2 *	15±2 *	51±14 *	11±4 *		
MDA-MB-468	5	69±12 *	47±2 *	53±8 *	100±7	82±4 *		
	10	56±18 *	41±4 *	48±11 *	100± 6	65±4 *	30±7 *	100±11
	20	50±8 *	33±2 *	36±9 *	77±12 *	51±7 *		

Values are mean±SD of percent cell survival, using MTT assay, n =6; [§] Doxorubicin (20 µg/ml) was used as positive control; [Φ] no drug was added; ∗= $P< 0.05$ compared to control group.

Cytotoxic effect of extracts against MDA-MB-468 cells

As shown in Table1, hydroalcoholic extracts of female branchlets (5, 10 and 20 µg/ml) and berries (10 and 20 µg/ml) of *J. excelsa* subsp. *polycarpos* were cytotoxic against MDA-MB-468 cell line, whereas with extracts of *J. excelsa* subsp. *excelsa* IC_{50} was not obtained.

Discussion

Isolation and identification of some potent anti-tumor compounds such as colchicine, *Vinca* alkaloids and recently paclitaxel (taxol®) from medicinal plants, has encouraged scientists to screen the effect of different parts of plant species on the cancer cells.

MTT-based cytotoxicity assay was used to evaluate the antiproliferation activity of tested plants. Our findings indicated that different parts of *J. excelsa* subsp. *polycarpos* and *J. excelsa* subsp. *excelsa* had various cytotoxic effects against Hela, KB and MDA-MB-468 cell lines. The most potent cytotoxic effect was seen for hydroalcoholic extracts of all tested parts of *J. excelsa* subsp. *polycarpos* against KB cells (IC_{50}<5 µg/ml), while *J. excelsa* subsp. *excelsa* extracts were cytotoxic only at concentrations≥ 20 µg/ml. It is worth to mention that extracts from branchlets and berries of *J. excels* subsp. *polycarpos* in concentration of 20 µg/ml was more effective than that of doxorubicin in the same concentration (Table 1). KB cell line is a human oral epithelial-like cell most commonly used as a model system in studies of biological activity of antineoplastic agents (12). It has been shown that these cells are sensitive or supersensitive in the presence of antineoplastic agents (13). Lower IC_{50} for this cell line in our studies is consistent with the above-mentioned findings (Table 1). Topcu and co-workers (14) showed that diterpenes and sesquiterpenes extracted from the berries of *J. excelsa* subsp. *excelsa* had cytotoxic activity against a panel of cell lines [human colon cancer cell line (LNCaP), KB-V (+VLB) and KB-V (-VLB)] and *Mycobactrium tuberculosis*. We found the same activity for *J. excelsa* subsp. *excelsa* against KB cells in our study. On the other hand, Lim and colleagues (15) reported that the methanol extracts of *J. chinensis* showed strong antioxidant activity, determined by measuring the radical scavenging effect on DPPH (1,1-diphenyl-2-picrylhydrazyl) which may be responsible for its cytotoxic effects. As hydroalcoholic extracts from branchlets and fruits of *J. excelsa* subsp. *polycarpos* had comparable cytotoxic effects with doxorubicin, it is worth to carry out further studies on the cytotoxicity of different fractions of this plant and identify its active components.

Acknowledgment

This study was supported by a grant from the Research Council of Isfahan University of Medical Sciences, Isfahan, Iran. The authors declare that they have no conflict of interests.

References

1. Adams RP. *Juniperus* of the world. Vancouer: Trafford Publishing Co; 2004.
2. Sabeti H. Forests, trees and shrubs of Iran. Tehran: Ministry of Information and Tourism Press; 1975.
3. Hooper D, Field H. Useful plants and drugs of Iran and Iraq. Field Museum of Natural History Botany 1937; 3:73.

4. Yesilada E, Honda G, Sezik E, Tabata T, Fujita T, Tanaka T, *et al.* Traditional medicine in Turkey V. Folk medicine in the inner Taurus mountains. J Ethnopharmacol 1995; 46:133-152.
5. Muhammad I, Mossa JS El-Feraly FS. Antibacterial diterpenes from the leaves and seeds of *Juniperus excelsa* M. Bieb. Phytother Res 1992; 6:261-264.
6. Singh V. Traditional remedies to treat asthma in North West and Trans Himalayan region in Jamoo and Kishmir state. Fitoterapia 1995; 695:507-509.
7. Sadeghi-aliabadi H, Emami SA, Saeidi M, Jafarian A. Cytotoxic effects of the extracts of Iranian *Taxus baccata* and *Cupressus horizentalis* on cancer cells. Iran J Pharm Res 2003; 2:107-110.
8. Jafarian-Dehkordi A, Emami SA, Saeidi M, Sadeghi-aliabadi H. Cytotoxicologic studies of the extracts of Iranian *Juniperus sabina* and *Platycladus orientalis* on cancer cells. J Res Med Sci 2004; 5:7-11.
9. Emami SA, Sadeghi-aliabadi H, Saeidi M, Jafarian A. Cytotoxic evaluation of Iranian conifers on cancer cells. Pharm Biol 2005; 43:299-304.
10. List H, Schmidt P Technologie Pflanzlicher Arzneizuberitungen, Wissenschafliche Verlagsgesellschaft mbH Stuttgart, 1984; p.140
11. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65:55-63.
12. DiRienzo JM, Song M, Wan LSY, Ellen RP. Kinetics of KB and Hep-2 cell responses to an invasive, cytolethal distending toxin-producing strain of *Actinobacillus actinomycetemcomitans*. Oral Microbiol Immunol 2002; 17:245-251.
13. Yen Y, Grill SP, Dutschman GE, Chang CN, Zhou BS, Cheng YC. Characterization of a hydroxyurea-resistant human KB cell line with supersensitivity to 6-thioguanine. Cancer Res 1994; 54:3686-3691.
14. Topcu G, Erenler R, Cakmak O, Johansson CB, Celik C, Chai HB, *et al*. Diterpenes from the berries of *Juniperus excelsa*. Phytochemistry 1999; 50:1195-1199.
15. Lim JP, Song YC, Kim JW, Ku CH, Eun JS, Leem KH, *et al*. Free radical scavengers from the heartwood of *Juniperus chinensis*. Arch Pharm Res 2002; 25:449-452.