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

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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/H2O (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

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**Cytotoxicity of Juniperus excelsa on Cancer Cells**

**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 subspecies: **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. excelsa** 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, IC50 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 (IC50<5 µg/ml; Table 1). For **J. excelsa** subsp. **excelsa** only the hydroalcoholic extracts of berries were cytotoxic against this cell line (IC50<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.

<table>
<thead>
<tr>
<th>Plants</th>
<th>J. excelsa subsp. polycarpos</th>
<th>J. excelsa subsp. excelsa</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Branchlets (male)</td>
<td>Branchlets (female)</td>
<td>Berries</td>
</tr>
<tr>
<td>Hela</td>
<td>5 87±7 *</td>
<td>80±8 *</td>
<td>105±19</td>
</tr>
<tr>
<td></td>
<td>10 70±12 *</td>
<td>69±2 *</td>
<td>63±3 *</td>
</tr>
<tr>
<td></td>
<td>20 58±3 *</td>
<td>43±16 *</td>
<td>46±16 *</td>
</tr>
<tr>
<td>KB</td>
<td>5 28±5 *</td>
<td>32±1 *</td>
<td>28±1 *</td>
</tr>
<tr>
<td></td>
<td>10 22±3 *</td>
<td>18±3 *</td>
<td>20± 6 *</td>
</tr>
<tr>
<td></td>
<td>20 7±2 *</td>
<td>10±2 *</td>
<td>15±2 *</td>
</tr>
<tr>
<td>MDA-MB-468</td>
<td>5 69±12 *</td>
<td>47±2 *</td>
<td>53±8 *</td>
</tr>
<tr>
<td></td>
<td>10 56±18 *</td>
<td>41±4 *</td>
<td>48±11 *</td>
</tr>
<tr>
<td></td>
<td>20 50±8 *</td>
<td>33±2 *</td>
<td>36±9 *</td>
</tr>
</tbody>
</table>

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 Table 1, 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 IC50 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 (IC50<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. excelsa 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 IC50 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

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References


Cytotoxicity of *Juniperus excelsa* on Cancer Cells


