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Cytotoxic effects of *Cuscuta* extract on human cancer cell lines

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Cancer is a growing health problem around the world. Although there are different therapeutic methods for cancer such as lymphoma and melanoma cancers, none of them have possessed complete efficacy up to now. Therefore, discovery of novel anti-cancer drugs is important. In this study, the cytotoxic effect of *Cuscuta* extract, a traditional Iranian medicinal herb, on melanoma cell line (SK-MEL-3) and human Burkitt lymphoma (Raji) is evaluated. MTT assay was performed for cytotoxic effect of *Cuscuta* extract. The most cytotoxic effects of *Cuscuta* extract on SK-MEL-3 and Raji cell lines were 80 and 81%, respectively, compared to a control group. According to our data, Raji cells are more sensitive to *Cuscuta* than the SK-MEL-3 cells. *Cuscuta* extract seems to be a good candidate as an anti-cancer agent against lymphoma and melanoma cancers. To clarify the effective molecules and their mechanisms, further studies are undertaken in our laboratory on animal models and humans.

Keywords: *Cuscuta*; melanoma cancer (SK-MEL-3); lymphoma cancer (Raji); cytotoxic

1. Introduction

Cancer is one of the most mortal diseases worldwide, and melanoma is the most aggressive form of skin cancer (Brohem et al., 2009; Dancey, Mahon, & Rayatt, 2008; Garbe & Leiter, 2009). Cutaneous melanoma has shown increasing incidence rates and has developed from a very rare disease entity into a cancer with growing importance medically and death (Chan, O'Donnell, Whitehead, Ryman, & Sullivan, 2007). Leukaemia and lymphomas are known as two of the most common malignant diseases affecting children (Pui, 2000). Global epidemiologic studies have demonstrated that the incidence and mortality rates of lymphoma still rank high in the worldwide population (Jemal et al., 2003). The incidence of lymphoma and melanoma continue to increase worldwide, whereas their effective treatment is limited. Unfortunately, investigations have shown that anticancer drugs are usually known to cause severe adverse effects and many complications such as suppression of the immune system, which has limited the use of anticancer drugs (Kaur, Michael, Arora, Härkönen, & Kumar, 2005; Liu et al., 2008).

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Herbal medicines are rich sources of natural anticancer materials (Tan & Vanitha, 2004) and thus good candidates for development of anticancer drugs. The worldwide-spread genus *Cuscuta* (*Cuscutaceae*) includes many species. *Cuscuta chinensis Lam* known as aftimun is one of the herbal medicines with many therapeutic effects. Some studies have reported that *Cuscuta* possesses a number of biological properties including: anticancer (Nisa, Akbar, Tariq, & Hussain, 1986; Umehara et al., 2004), immunostimulatory and antioxidant (Bao, Wang, Fang, & Li, 2002) activities. It has also been applied to improving the liver, kidney and vision complications (Yen, Wu, Lin, & Lin, 2007).

Regarding the investigations carried out for detection of potent and selective anticancer compounds from natural products, the anticancer effects of *Cuscuta* extract were studied against two cancer cell lines SK-MEL-3 and Raji at various concentrations. According to our knowledge, there have been few reports on the effect of *Cuscuta* on various cancer cell lines; particularly, there has been no report on SK-MEL-3 and Raji cell lines. In the present study, we report the anticancer activity of *Cuscuta* aqueous extract on these cell lines.

2. Materials and methods

2.1. Preparation of Cuscuta *extract*

Cuscuta was processed in the Pharmacological Department of Iranian Traditional Medicine, Shahed University, with voucher Number PMP_303 in medicinal plant herbarium of Tehran University. For preparation of *Cuscuta* extract, 100 g of the aerial part of *Cuscuta* was put into boiling water, the solution was filtrated and dried extract used. All extracts were used in different concentrations (5, 2, 1, 0.2. 0.1, 0.05, 0.02 and 0.01 mg/ml).

2.2. Chemicals

RPMI-1640 medium and foetal bovine serum (FBS) were obtained from Gibco. Penicillin–streptomycin was obtained from Sigma-Aldrich, USA. MTT [3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] powder and phosphate buffer saline (PBS) were obtained from Merck (Germany). Human malignant melanoma (SK-MEL-3) cell line and human Burkitt lymphoma cell line (Raji) were purchased from the Pasteur Institute, Tehran, Iran. Peripheral blood mononuclear cells (PBMC) were obtained from the peripheral blood of healthy volunteers after centrifugation on a Ficoll gradient (Moosavi, Yazdanparast, Sanati, & Nejad, 2005).

2.3. Cell culture

Malignant and non-malignant cells were cultured in RPMI-1640 medium (Gibco) containing 10% serum FBS (Gibco) and 100 IU/ml penicillin–streptomycin (Sigma) and incubated at 37°C in a humidified atmosphere in the presence of 5% CO_2.

2.4. Viability assay

MTT reduction assay was used for assessing cells viability. Briefly, MTT powder was dissolved in PBS. Cells were seeded at 10,000/well onto 96-well culture plates and allowed to grow for 24 hours. Then cells were treated with different concentrations (5–0.01 mg/ml) of *Cuscuta* extract and for different periods of time including 24, 48 and 72 h. Four hours before the end of each time period, 20 µl of MTT solution (5mg/ml) was added to each culture. MTT was converted by intact mitochondrial reductase and precipitated as blue crystals during a four hour contact period. The supernatants were gently removed and the formazan crystals were resolved in 100 µl acidic isopropanol (0.04 M HCl in isopropanol), and absorbance was read at 540 nm with a plate reader (Stat-Fax 2100, USA) (Abuharfeil, Al-Oran, & Abo-Shehada, 1999; Lee, Kang, Hwang, & Kim, 2011).

Data were presented as proliferation inhibitory rate of cell, the formula is: cell proliferation inhibition rate (%) = (1–OD value of drug treatment group/normal medium control OD value).

2.5. Statistical analysis

The results are presented as mean ± SEM. Analysis of variation was done and comparisons between study groups were performed with ANOVA and students *t*-test. Differences were considered significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$.

3. Results

3.1. Effect of Cuscuta *extract on cell viability of SK-MEL-3*

As our results show, cells treated with high concentrations (5, 2 and 1 mg/ml) of *Cuscuta* extract significantly decreased cell viability at 24, 48 and 72 h versus control group. Whereas concentrations of 0.05 and 0.01 mg/ml of *Cuscuta* extract increased viability of the cells at 24 h, other doses induced no significant differences on cell viability in comparison with control group (Figure 1).

3.2. Effect of Cuscuta *extract on cell viability of Raji*

Results presented in Figure 2 show that cell viability of Raji lymphoma cell line is significantly decreased by high concentrations (5, 2 and 1 mg/ml) of *Cuscuta* extract at 24, 48 and 72 h. Furthermore, viability of the cells decreased significantly ($P < 0.05$) at 48 h by using concentrations of 0.5, 0.1, 0.05 and 0.01 mg/ml of *Cuscuta* extract (Figure 2).

3.3. Effect of Cuscuta *extract on PBMC*

As our results show, cells treated with a concentration of 5 mg/ml of *Cuscuta* extract significantly decreased cell viability at 24, 48 and 72 h versus control group, whilst a concentration of 2 mg/ml significantly decreased cell viability at 24 and 48 h in comparison with the control group (Figure 3).

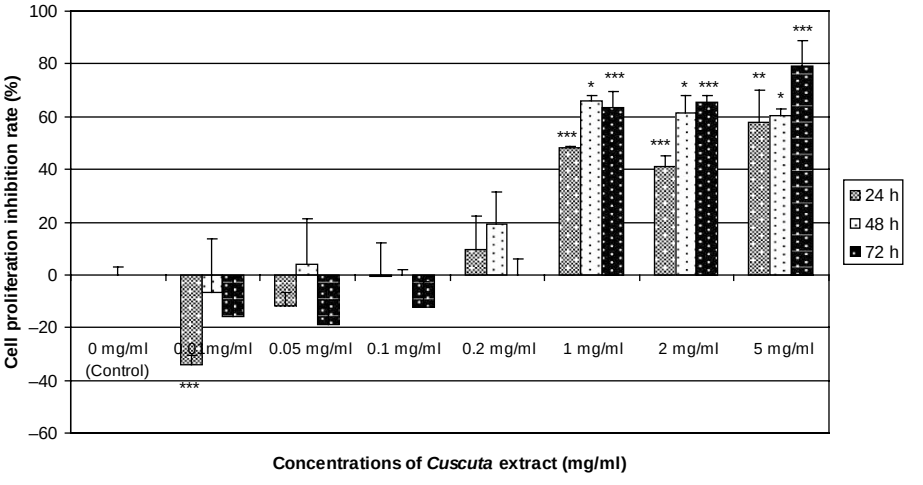

Figure 1. Comparison of cell proliferation inhibition rate (%) of *Cuscuta* extract on SK-MEL-3 melanoma cell line with different concentrations for 24, 48 and 72 h. * denoted significant differences. $*P<0.05$, $**P<0.01$ and $***P<0.001$ compared to control.

3.4. Comparison of cytotoxic effects of Cuscuta *extract on malignant (SK-MEL-3 and Raji) and non-malignant cells*

Results provided in Figure 4 indicate that *Cuscuta* extract decreased cell viability in malignant cells but not in non-malignant cells at 72 h. Doses inducing 50% cell growth inhibition (IC_{50}) against SK-MEL-3 and Raji cells and selectivity index (SI) are given in Table 1.

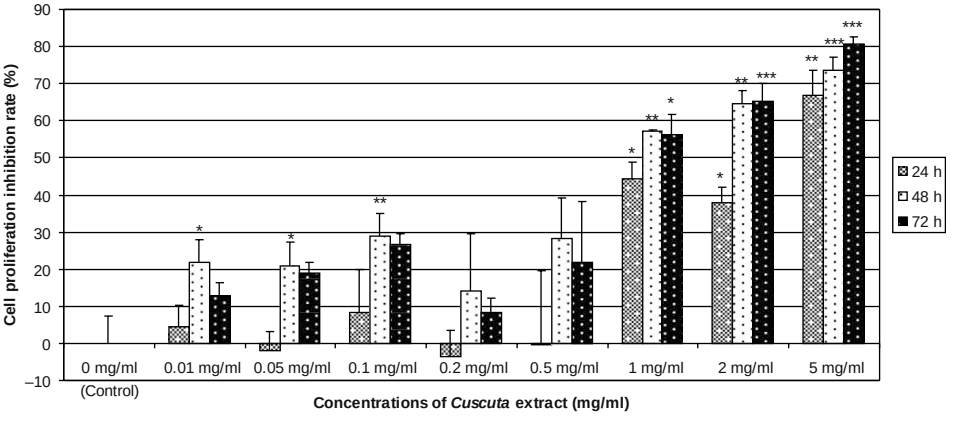

Figure 2. Comparison of cell proliferation inhibition rate (%) of *Cuscuta* extract on Raji lymphoma cell line with different concentrations for 24, 48 and 72 h. * denoted significant differences. $*P<0.05$, $**P<0.01$ and $***P<0.001$ compared to control.

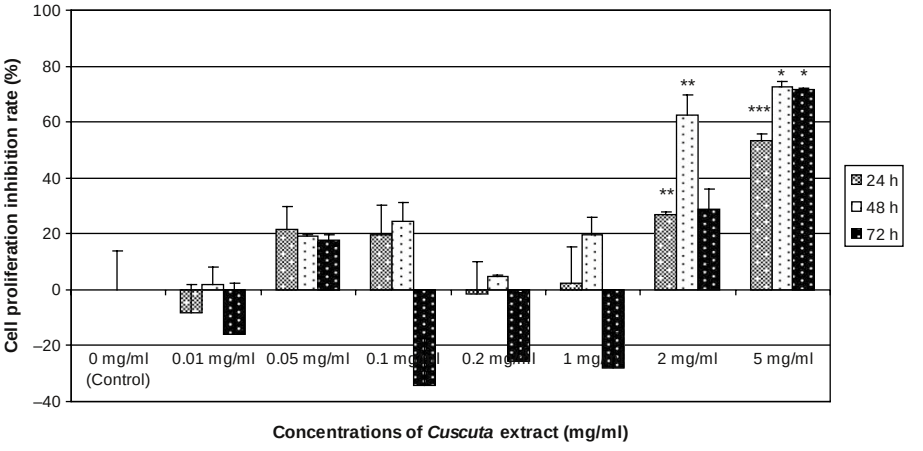

Figure 3. Comparison of cell proliferation inhibition rate (%) of *Cuscuta* extract on peripheral blood mononuclear cell with different concentrations for 24, 48 and 72 h. * denoted significant differences ($P < 0.05$). *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared to control.

4. Discussion

Cancer is the second leading cause of death in many countries worldwide (Kirman et al., 2007) and is one of the major problems in the present century. Melanoma and lymphoma are malignancies for which no effective treatments have been found up to now. Plants have been important resources in traditional medicine (Tavakkol-Afshari, Brook, & Mousavi, 2008). The main advantages of natural bioactive molecules include their mild side effects on the body in comparison with chemically synthesised drugs. Regarding many problems with the use of chemical drugs for

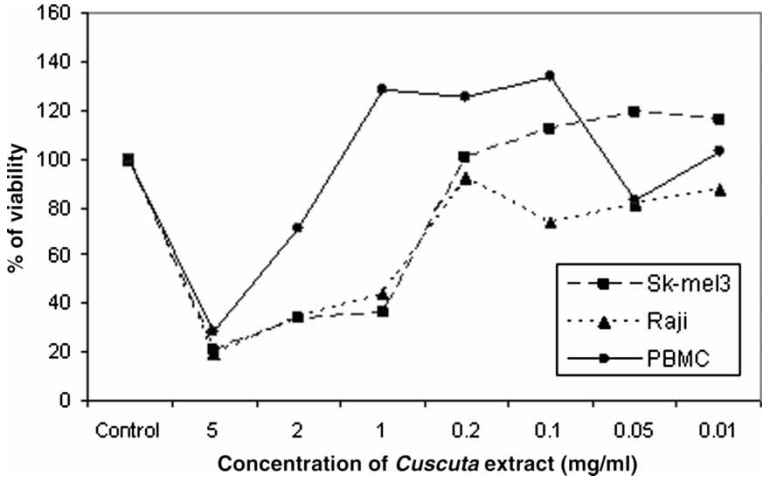

Figure 4. Comparison of cytotoxic effects of *Cuscuta* extract on malignant (SK-MEL-3 and Raji) and non-malignant (PBMC) cells. Cells were treated with different concentrations of *Cuscuta* extract for 72 h.

Table 1. Doses inducing 50% cell growth inhibition (IC_{50}) of *Cuscuta* extract against SK-MEL-3 Raji and PBMC cell lines. Selectivity index (SI) is the ratio of IC_{50} normal cells to cancer cell lines.

	24 h		48 h		72 h	
Duration time of cell culture	IC_{50} (mg/ml)	SI	IC_{50}	SI	IC_{50}	SI
SK-MEL-3	3.534	0.001	2.888	0.94	2.56	1.49
Raji	2.95	1.59	2.064	1.31	2.060	1.86
PBMC	4.708		2.723		3.833	

cancer treatment, attention has shifted to the discovery of new anticancer drugs from herbal medicines. *Cuscuta* is one of the effective natural products with anticancer (Umehara et al., 2004) and an immunostimulatory effect (Bao et al., 2002).

Since it is known that different cell lines might exhibit different sensitivities when they are exposed to one cytotoxic compound, the effect of a product must therefore be examined in different cells. On the other hand, no direct published data are available on the cytotoxic effect of *Cuscuta* on melanoma and lymphoma cancer. So in this study, the cytotoxic effect of *Cuscuta* extract on viability of SK-MEL-3 and Raji cell lines was investigated.

Our results revealed that the most cytotoxic effect of *Cuscuta* extract on SK-MEL-3 cell line is 59% achieved at a concentration of 5 mg/ml at 24 h. A concentration of 1 mg/ml of *Cuscuta* extract has the highest cytotoxic effect (66%) on SK-MEL-3 cell line at 48 h. In 72 h culture, a concentration of 5 mg/ml led to the highest cytotoxic effect (80%) on SK-MEL-3 cell line.

In this study, a concentration of 5 mg/ml of *Cuscuta* extract resulted in the highest cytotoxic effect (67%) on Raji cell line at 24 h. Also, a concentration of 5 mg/ml of *Cuscuta* extract has the highest cytotoxic effect (74%) on Raji cell line at 48 h. The maximum cytotoxic effect of *Cuscuta* extract on Raji cell line at 72 h was achieved at a concentration of 5 mg/ml (81%). However, the cytotoxic effect of *Cuscuta* extract on Raji cell line starts from 0.01 mg/ml at 48 h after exposure and reaches 68% after the use of 1 mg/ml of *Cuscuta* extract.

In the case of PBMCs there is no significant toxicity until the use of 2 mg/ml and this indicates that the best dose is 1 mg/ml, which is toxic for cancerous cell lines and is safe for PBMCs.

In the present study, the IC_{50} value of *Cuscuta* extract on SK-MEL-3, Raji and PBMC cells at 72 h was 2.56, 2.06 and 3.833 mg/ml, respectively. Our data suggest that Raji cells are more sensitive to *Cuscuta* than the SK-MEL-3 cells.

According to the report by Nisa et al. (1986), anti-tumour activity of different *Cuscuta* species was shown in skin carcinoma in Swiss albino mice. Although model examination in the study by Nisa et al. was different from that of our study, the results of the present study are in agreement with their findings. Yen, Wu, Lin, Cham and Lin (2008) reported that *Cuscuta chinensis* extract and its fractions possess antioxidant activities for preventing free radical damage to cell membranes (Bao et al., 2002; Wang, Fang, Ge, & Li, 2000). Wang et al. (2000) reported that an acidic polysaccharide, isolated from the seeds of *Cuscuta chinensis*, remarkably promoted the proliferation of T-cells and B-cells in vitro (Pan, Sun, & Pan, 2005). Pan et al. (2005) reported that an ethanol extract of semen Cuscutae (the dry seed of *Cuscuta*

chinensis) is effective on Th1 and Th2 cell functions (Yen et al., 2008). Considering that the antioxidant and immunomodulatory effects are highly desirable in cancer treatment, these properties, in association with the cytotoxicity effects, have made *Cuscuta* a valuable goal to achieve an anticancer drug. Interestingly, in the present study, low concentrations of *Cuscuta* exert stimulatory effects on SK-MEL-3, more studies on its fractions and isolation of its various effective materials are needed to clarify this result, one explanation is that *Cuscuta* extract have probably also included a stimulatory fraction which show its effects at the absence or low doses of toxic agents.

Further investigations in animal models are needed to clarify other mechanisms for cytotoxic and anti-tumour actions of *Cuscuta* extract on SK-MEL-3 and Raji cell lines in humans.

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