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Original Article

In Vitro Cytotoxic Effects of *Cuscuta chinensis* Whole Extract on Human Acute Lymphoblastic Leukemia Cell Line

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Abstract

Background: One of the major paths for drug development is the study of bioactivities of natural products. Therefore, the aim of this study was to compare the cytotoxic effects of aqueous extract of whole *Cuscuta chinensis* Lam., which is a traditional medicinal herb commonly used in Iran and other oriental countries, on the human caucasian acute lymphoblastic leukemia (CCRF-CEM) and another human lymphocyte, Jurkat (JM) cell lines.

Methods: *In vitro* cytotoxic screening with various concentrations (0, 0.1, 1, 10, 25 and 50 µg/ml) of the extract was performed using microscope and methyl tetrazolium bromide test (MTT).

Results: The minimum effective concentration of the plant extract was 1 µg/ml, and increasing the dose to 10 µg/ml induced increasingly stronger effects. The inhibitory concentration 50% (IC_{50}) of the extract against CCRF was about 3 µg/ml in 24 hours and 2.5 µg/ml in 48 hrs. In contrast, the extract did not have cytotoxic effect for the JM cells at these doses.

Conclusion: The findings of the present study suggest that *C. chinensis* is toxic against CCRF-CEM and JM tumor cells. Whether or not such effects can be employed for the treatment of such tumors must await future studies.
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Keywords ● *Cuscuta* ● acute lymphoblastic leukemia ● antitumor agent ● MTT formazan

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Introduction

Nowadays, the use of traditional herbal medicine as alternative treatment has been revived all over the world. People have started to use some of the commonly well-known herbs crude extract as daily food supplement and tonic. However, the effects of a lot of such herbs on the body system have not been documented. Therefore, researchers have started to study the biological properties of the traditional herbs.[1-3] One of these herbs is *Cuscuta chinensis* Lam., which is a delicate, twining, glabrous, yellowish-green, and thread-like parasitic herb belonging to the family *Convolvulaceae*, which is distributed in tropical and temperate regions. The herb is known as Aftimun in India's traditional medicine.[4,5]

Cuscuta chinensis is an important herbal medicine that is used as tonic and aphrodisiac to nourish the liver and kidneys, and to treat impotence and seminal emission.[6,7] Moreover, it is considered to have antitumor effects in prostate cancer, skin carcinomas and papillomas,[5,8] antimutagenic activity against heterocyclic amines, cytotoxicity effect for lymphoblastic- like tumor cell line (HL60),[9] and immunostimulating and antioxidant functions.[6, 10] Although, several studies,[5,8,9] have demonstrated the biological functions of *C. chinensis* as antitumor, a number of other observations showed that *C. chinensis* stimulated the proliferation of epidermoid carcinoma (MCF-7) cell line, human breast cancer (T47D) cell line,[11] and lymphocytes and rat bone cells.[10,12] In addition, Aftimun was shown to activate and differentiate human osteoblast-like MG-63 cells and rat pheochromocytoma PC12 cells.[13,14]

There is, however, no direct experimental evidence for the cytotoxic effect of aqueous extract of whole *C. chinensis* on tumor cells. Therefore, the present study was designed to investigate *in vitro* cytotoxic effect of aqueous extract from whole *C. chinensis* on human caucasian acute lymphoblastic leukemia (CCRF-CEM) and a human lymphocyte, Jurkat (JM), cell lines using methyl tetrazolium bromide test (MTT).

Materials and Methods

Preparation of the Plant Extract

Seeds of *C. chinensis* were purchased from a local herbal medicine grocery (Hamadan, Iran) and authenticated by one of the authors; KR. The seeds were grown on *Alyssum campestre L.*, and the whole part of the fresh *C. chinensis* was used to prepare the aqueous extract. Two hundred grams of the plant were mixed with 50 ml of distilled water, and the extract obtained through pressing and grinding of the plant using stainless steel press. The extract was filtered twice using a Whatman filter paper, and then sterilized with a 0.2 µm filter. The moisture of the extract was determined by placing 4 grams of the final extract in an oven at 50°C for 2 h. The extract was then weighed, and the weight loss was used as a moisture indicator. The final extract contained 88 mg/ml of the plant.

Cell Lines

CCRF-CEM and JM cell lines were obtained from the National Cell Bank of Iran (NCBI) at Pasteur Institute. The cells were maintained, cultured and incubated in $RPMI_{1640}$ medium (Gibco-BRL, Australia) with 100 U/ml penicillin G (Hayan, Iran), 10% FCS (Gibco-BRL, Australia), 100 µg/ml streptomycin (Hayan, Iran) at temperature of at 37ºC, a CO2 of 5%, and a humidity of 90 % throughout the study. The cells viability was assessed by the trypan blue exclusion method.[2]

Microscopic analysis

CCRF-CEM and JM cells were seeded into the 6-well plates. Each well contained 5×10^4 cells/ml, which were incubated for 4 h to allow their adaptation and treated with the various concentrations (0, 0.1, 1, 10, 25 and 50 µg/ml) of the plant extract for 24 and 48 hours. After the incubation of the cells with the extract, morphological changes and granularity of the cells were examined by an inverted microscope.[14]

Methyl tetrazolium bromide Cytotoxicity Assay

CCRF-CEM or JM cells ($n=2.5\times10^5$ cells) were seeded into the 12-well plates, and incubated with 0.5 ml medium per each well for 4 hours. Then, cells were exposed to the different concentrations (0, 0.1, 1, 10, 25 and 50 µg/ml) of the plant extract for 24 and 48 h. After the incubation of the cells with the extract, each vial of MTT reagent (Sigma, USA) was reconstituted with 3 ml of phosphate buffer solution (PBS) with a pH=8, and added in an equal volume to 10% of the culture medium. The cells were returned to incubator for 4 hours. Then, the plates were removed from the incubator and the resulting formazan crystals were dissolved by adding MTT solubilization solution in equal volume of the original culture medium. MTT formazan crystals were completely dissolved by pipetting up and down. The absorbance was measured at the wavelength of 570 nm,[15] using UV-vis spectrophotometer (Spectronic Genesys, USA). The cells' viability was determined according to the optical density (OD) of the wells which contained no extract. The inhibitory concentration 50% (IC_{50}) was defined as the minimum concentration of the extract that reduced viability of the incubated cells after 24 and 48 h by 50%.[2,9]

Results

The CCRF-CEM and JM cell lines incubated with *C. chinensis* extract showed a low growth rate. Compared to JM cells, the CCRF-CEM cell line showed a significant increase in death and granularity (figure 1). As shown in figure 2, the viability of the CCRF-CEM cells was also reduced compared to the JM cells. The minimum effective concentration for the plant

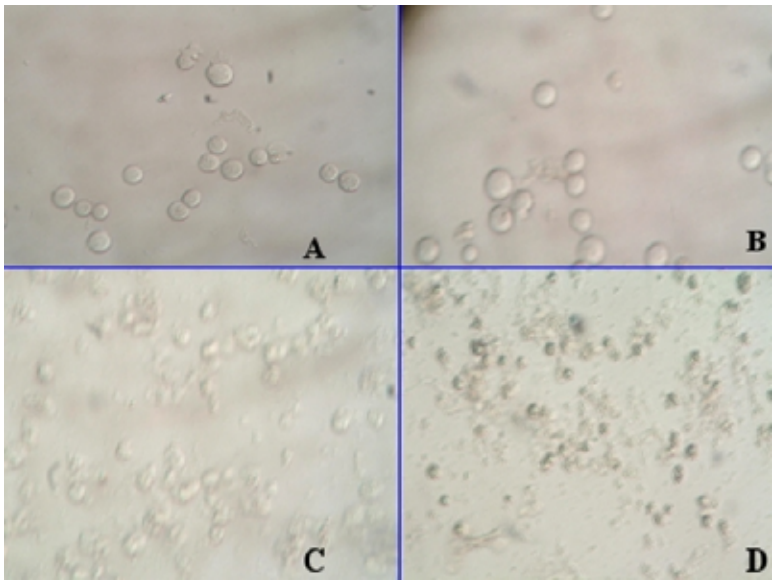

Figure 1: Morphological analysis of the cell lines. A and B; human lymphocyte, Jurkat cell line (JM), and C and D; human caucasian acute lymphoblastic leukemia (CCRF-CEM) before and after treatment with 10 µg/ml of aqueous extract for 24 hours (Magnitude 400X).

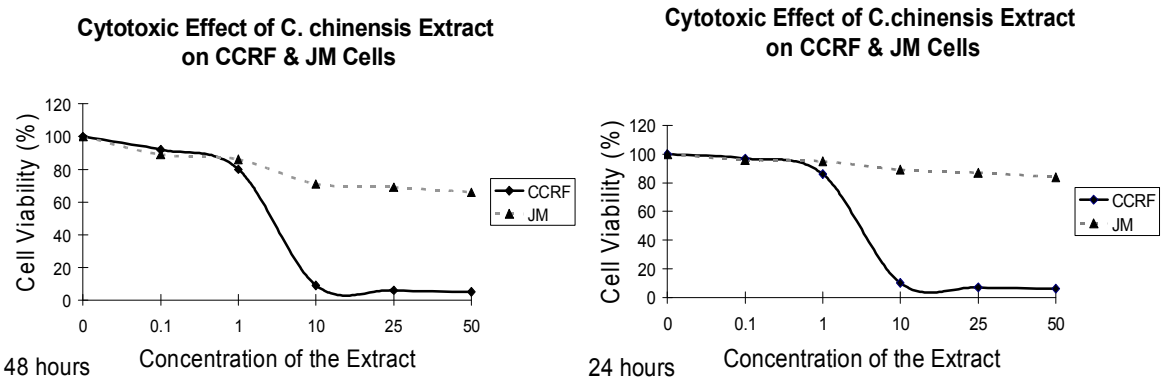

Figure 2: The cytotoxic effect of various concentrations of C. chinensis extract after 24 and 48 hours incubation on the lymphoblastic leukemia (CCRF-CEM) and a human lymphocyte, Jurkat (JM), cell lines. The cells (2.5×10^5) were cultured on each wells of 12-well plates for 4 hours, and were then exposed to various concentrations of C. chinensis extract for 24 and 48 h. Methyl tetrazolium bromide test (MTT) assay was used to check the cells viability and the extract had inhibitory concentration 50% (IC_{50}) about 3 µg/ml in 24 h and 2.5 µg/ml in 48 h incubation for CCRF. The extract did not display strong cytotoxic effect on JM cells, and had no IC_{50} that could be obtained at the range of concentrations used. Data were the average of two different independent experiments.

extract was 1 µg/ml, and increasing the dose to 10 µg/ml showed increasingly stronger effects. As shown in figure 2, IC_{50} of the extract for CCRF was 3 µg/ml after 24 h and 2.5 µg/ml after 48 h incubation. The cytotoxic effect of the plant extract was not significantly different after 24 or 48 h of incubation.

The extract did not reduce the viability of JM cells, and did have no IC_{50} that could be determined at the range of used concentrations of the extract.

Discussion

To evaluate the cytotoxic effect of the aqueous extract of whole *C. chinensis* on two cell lines, the viability of the human caucasian acute lymphoblastic leukemia (CCRF-CEM) and a human lymphocyte (JM) cell lines were tested using MTT assay. The significant reduction of CCRF-CEM cell line viability compared with JM cells showed that the extract induced cytotoxicity on CCRF-CEM with minimal effect on other

human lymphocyte cell line. To our knowledge, there is only one somehow similar research which reported that the methanolic extract of *C. chinensis* had cytotoxic effect against a tumor cell line, HL60, with an inhibitory concentration 100% (MIC_{100}) of 4.0 µg/ml.[9] Therefore, the potent cytotoxic activity of the aqueous extract of whole *C. chinensis* is reported for the first time in this study. Our results are also generally in agreement with those of a published study,[5] which showed that the oral administration of a hot water extract of *C. chinensis* whole plant (1 g/kg body weight) thrice a week on 7, 12-dimethylbenz[a]anthracene (DMBA)-induced skin papillomas and carcinomas in Swiss albino mice markedly delayed the appearance and retarded the growth of papillomas and the incidence of carcinoma, relative to a control group. Moreover, the findings of the present study are similar to those studies, which demonstrated that equiguard, a dietary supplement comprised of standardized extracts from nine herbs including *C. chinensis* (ethanol extract of seed), significantly reduced prostate carcinoma cell growth,[8] induced apoptosis,[8] showed antioxidant activity,[6] and indirectly stimulated the antitumor activity.[16,17]

The results of this study are, however, in contrast with those of a number of previous studies.[10-12] When added to the bone cell culture, crude extract of *C. chinensis* promoted the proliferation and differentiation of osteoblasts from their precursor cells.[12] Moreover, cuscutic resinoside A, a resin glycoside isolated from the seeds of *C. chinensis* showed potency for stimulating MCF-7 and T47D human breast cancer cells at a concentration of 10 µM.[11] As well, three crude polysaccharide fractions prepared from the seeds of *C. chinensis* by hot water extraction showed a stimulating effect on concanavalin A or lipopolysaccharide induced mitogenic activity of lymphocytes.[10]

The pharmacological effects of *C. chinensis* have been attributed to the main constituents of *C. chinensis* including flavonoids, saccharide, alkaloids, lignans, and resin glycosides.[10,11,18,19] It seems that the exact component(s) responsible for the cytotoxic effects may be found in the both aqueous and alcoholic extracts of the whole part of the plant. As evidence from this and others experimental studies,[5,8,9] suggest the exact component might be mahanine.

Mahanine is an interesting food component exhibiting a wide variety of bioactivities like cytotoxicity against HL60 cells.[9] It is expected that *C. chinensis* may contain mahanine and contribute to increase cytotoxicity and reduce cancer risks. To elucidate whether or not *C. chinensis* contains this component further investigations are required.

Conclusion

The findings of the present study suggest that *C. chinensis* is toxic against CCRF-CEM and JM tumor cells. Whether or not such effects can be employed for the treatment of such tumors must await future studies.

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Conflict of Interest: None declared

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