

Cytotoxic activity of *Isatis campylocarpa*, an Iranian endemic plant, on human cancer cell lines

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Abstract

The antineoplastic activities of alcoholic and aqueous extracts of the roots, stems and leaves of *Isatis campylocarpa* an endemic species of the Brassicaceae family from Iran, investigated on the growth of Jurkat as an acute lymphocytic leukemia cell line, K562 as a chronic myelogenous leukemia cell line and Fen as a bladder cancer cell line using colorimetric assay. Results showed that 1 to 200 µg/ml concentrations of all the extracts inhibited the proliferation of the cells and may be it relate to the Indirubin compounds. The maximum effect on the Jurkat cells observed for the aqueous root extract. The effect of the extracts on the Jurkat cells was greater than on the K562 cells, which may be indicate more sensitivity to lymphocytic cells than myeloid ones.

Key words: cytotoxic, *Isatis campylocarpa*, cancer, leukemia, indirubin

Introduction

Antineoplastic drugs prevent cancerous cell division in chemotherapy. Inhibition efficiency depends upon drug type and concentration. Chemotherapy has several side effects but natural compounds use in medicine, with fewer side effects. Sixty percent of antitumor and anti-infection drugs existing in market or under clinical studies have natural origins. Most of these compounds can not be synthesized chemically and they should be extracted from plants or produced by cell cultures (King and Robins, 2006; Asghari, 2006).

Isatis is a plant genus of the Brassicaceae family mainly distributed in the Irano-Turanian region and only a few species of them distributed in the Europe-Siberia and the Mediterranean phytogeography regions (Sajedi et al., 2005). One species of the genus i.e. *Isatis tinctoria* has been used in China as traditionally medicine in chronic myelogenous leukemia treatment (Xiao et al., 2002).

Indirubin, a compound found in *I. tinctoria* roots, has undergone screening for anti-cancer activity. Indirubin thought to be inhibiting DNA replication in neoplastic cells without causing significant bone

marrow suppression (Hoessel et al., 1999). The *Isatis* contains a number of indole compounds, which thought to have anti-cancer effects and may be help to explain the traditional uses of *Isatis* in the treatment of cancer. Indirubin competes with ATP for binding to catalytic sites of cyclin dependent kinases (CDKs) and block cell proliferation in the late-G₁ and G₂/M phases of the cell cycle, as well inhibits the assembly of microtubules. The crystal structures of CDK in complex with two Indirubin derivatives reveal the atomic interactions of these inhibitors with the kinases ATP-binding site (Hoessel et al., 1999; Meijer et al., 2006).

The MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) is a standard colorimetric assay to determine cytotoxicity of potential medicinal agents (Hay, 1988). MTT is water-soluble and reduced in living cells because mitochondrial reductase enzyme (dehydrogenase) activate and convert MTT into the purple crystals of formazan. These crystals are insoluble in water and their amounts will be directly related to the quantity of living cells (Masmann, 1983; Freshney, 1992).

The aim of the present study is to investigate the cytotoxic activity of the alcoholic and aqueous extracts of the roots, stems and leaves of *Isatis campylocarpa*, an Iranian endemic species.

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Material and Methods

Preparation of the extracts: Appropriate amounts of fresh materials of *I. campylocarpa* obtained from the north of Fars Province in Iran. A voucher specimen deposited in Shiraz University Herbarium. Different parts of the plant including the roots, stems and leaves completely separated and dried by air in the shade, powdered and subjected to extraction (both aqueous and alcoholic extracts). Different concentrations of aqueous extracts using acidic (pH=3) solution of RPMI 1640 medium (Sigma, St, Louis, USA) and alcoholic extracts using dimethyl sulphoxide (DMSO) were prepared from stock samples (20 mg/ml). Germinated seedlings as well used for aqueous extract. The solutions were centrifuged (3000 rpm for 10 min) to remove insoluble ingredients, and the supernatants passed through 0.22 µm filters for sterilization.

Cell lines: Suspension culture of Jurkat (T cell leukemia) and K562 (myelogenous leukemia) cell lines and monolayer culture of Fen (bladder carcinoma) cell line used. The cell lines obtained from an Iranian cell bank. All the cell lines were kept in RPMI 1640 medium (Sigma, St, Louis, USA) supplemented with 10% fetal calf serum (Gibco, Germany) in culture flasks at 37°C in 5% humidified CO₂ incubator. The cells fed until confluence (2×10⁶) and were expanded by trypsinization (for adherent cells) and subcultured at lower numbers in new culture flasks. Cells viability determined by trypan blue dye test.

MTT colorimetric assay: Briefly, 90 µl of each cell suspension containing 40000 Jurkat cells and 20000 K562 cells separately added into the 96 well culture plates. Then 10 µl of various prepared concentrations (2000, 1000, 500, 100, 10 µg/ml) from stock samples of the extracts were added to each row of wells and so the final concentrations of the extracts reached to 200, 100, 50, 10 and 1 µg/ml in each well. We used 10 µl of DMSO or acidic RPMI as negative control and 10 µl of doxorubicin (an anticancer agent) as positive control. In the case of monolayer cultures, 90 µl of cell suspension containing 15000 Fen cells placed into the wells, and incubated in CO₂ incubator for 24 h at 37°C and subsequently plant extracts added. Plates of suspension and monolayer cultures placed in the CO₂ incubator at 37°C for 48 h at humidified atmosphere. After the addition of 10 µl of MTT solution to each well, the plates transferred to the incubator and retained for 3-4 h. Supernatant removed only from the monolayer culture and then 100 µl of DMSO solutions added for dissolving of purple crystals of formazan, however in suspension cultures the supernatant did not remove. Then, 100 µl acidic isopropanol was added and the plates were placed in an incubator (5 min at 37°C) to remove bubbles from wells. Absorbance of each well was measured using ELIZA reader at 570 nm and 630 nm wavelength.

Analysis: Growth inhibition percentage (Inh%) for each concentration of the extracts was calculated as:

$$\text{Inh \%} = \left(1 - \frac{\text{Mean of light absorptions for test samples}}{\text{Mean of light absorptions for negative control}} \right) \times 100$$

The 50% inhibitory concentrations (IC₅₀) estimated by probit analysis (Minitab statistical software 13.30; Minitab Inc.). The assays carried out with three replications. Raw data imported to Microsoft Excel for calculations and graphical representation. SPSS version 11.5 and ANOVA used for analysis of variance and comparison of means by Duncan's method at $P < 0.05$.

Results

According to National Cancer Institute (NCI) protocol, significant cytotoxic activity of different extracts interpreted by IC₅₀ (Caideron, 2003). Cytotoxic activity will be considerable if IC₅₀ is

lower 25 µg/ml and will be weak if IC₅₀ is in the range of 25-100 µg/ml. The extract has no significant toxicity if IC₅₀ is more than 100 µg/ml. Therefore, with respect to this guideline, root aqueous extract of *I. campylocarpa* has a considerable toxicity (IC₅₀=10.2 µg/ml) and leaf aqueous extract (IC₅₀=50.1 µg/ml) has a weak cellular toxicity on Jurkat cells. In the case of Fen cells, root aqueous extract (IC₅₀=72.44 µg/ml), root alcoholic extract (IC₅₀=39.8 µg/ml), stem alcoholic extract (IC₅₀=97.7 µg/ml), and leaf alcoholic extract (IC₅₀=85.11 µg/ml) has weak cytotoxic activity. According to IC₅₀ value, other extract samples did not show significant toxicity. IC₅₀ values of three examined cell lines which

affected by different extracts are showed in Table 1. Inhibitory effects of different aqueous and alcoholic extracts of root, stem and leaf on Jurkat, K562 and Fen cell lines showed in Figure 1.

Discussion

Some researches have demonstrated that plant extracts possess various biological activities including anti tumor and anti leukemia activity (Tsan et al., 2002; Valente et al., 2004; Moongkarndi, 2004; Kanadaswami et al., 2005; Vasilev, 2005; Amirghofran et al., 2006). Therefore, plant substances continue to serve as viable source of anticancer drugs for the world population and several plant-based anticancer drugs are in clinical use (Heinrich et al., 2006). In the present study alcoholic and aqueous extracts of the roots, stems and leaves of *Isatis campylocarpa*, an Iranian endemic species, examined for cytotoxic activity against different cancerous cell lines. We found that based on IC₅₀, all of the extracts except stem aqueous extract and seedling aqueous extract more or less have the capacity to decrease the proliferation of cancerous cells. Among the plant extracts, the highest activity against the Jurkat cells was root aqueous extract and against the K562 cells was root alcoholic extract. The sensitivity of the Jurkat cells was more than K562 cells, which may be indicates more sensitivity of lymphocytic cells than myeloid ones and may be it relate to Indirubin compounds. Average inhibition of Jurkat, K562 and Fen cells treated with alcoholic and aqueous extracts of the different parts of *Isatis campylocarpa* was 130.6, 178.4 and 104.4 based on IC₅₀ and so Fen cells are more sensitive to this plant extracts.

Isatis tinctoria has used in China as traditionally medicine in chronic myelogenous leukemia treatment (Xiao, 2002), but the results presented, indicate that the extracts of *Isatis campylocarpa* used in this study have low antineoplastic activities against the cells that originated from leukemia. Leukemia is one of the most common malignancies causing death worldwide, and, although chemotherapy is the standard method of treatment for leukemia patients but is very expensive and has

not been fully effective, and therefore studies on another species of *Isatis* plant in Iran remains important to reduce the rate of mortality.

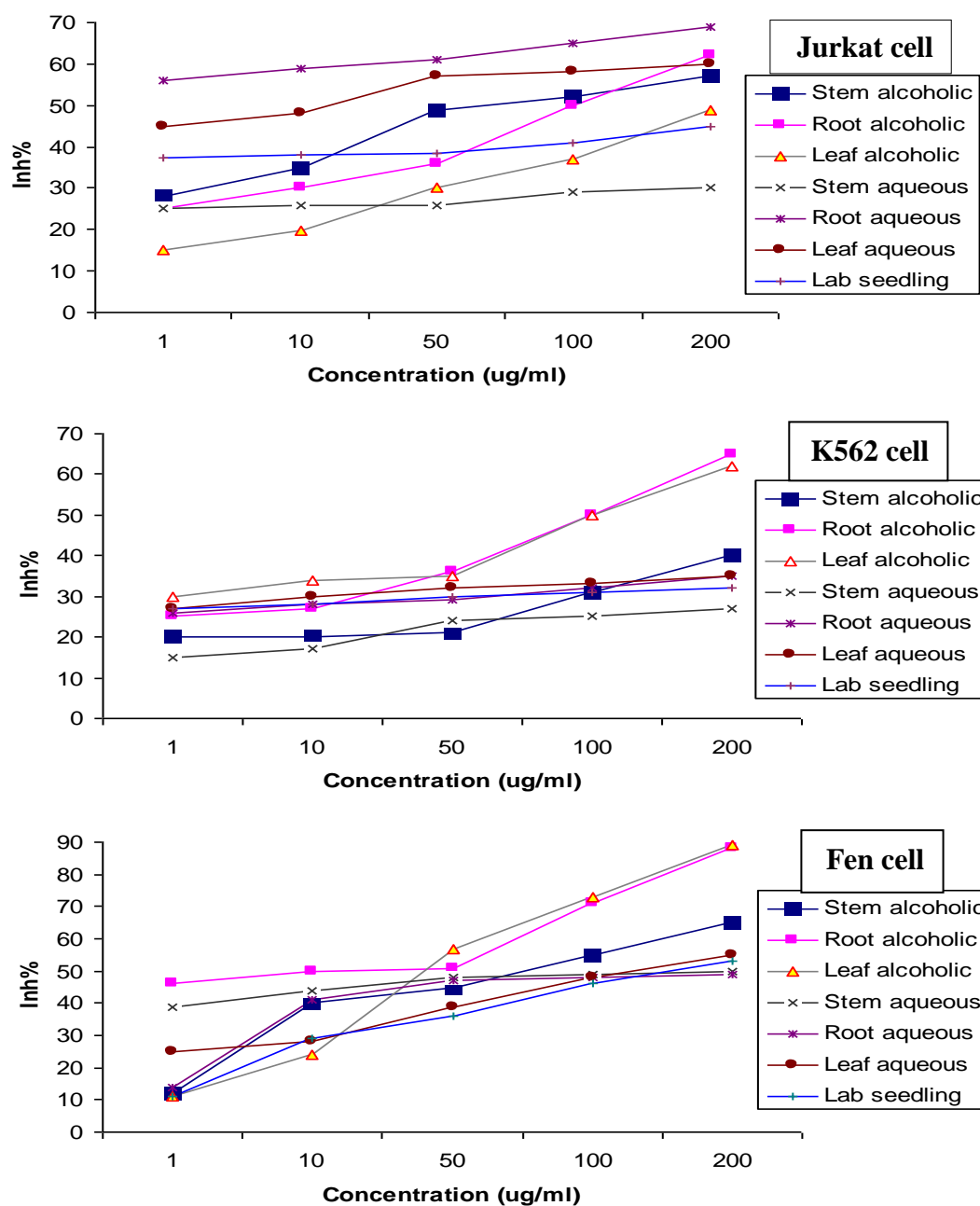
It has showed that the mechanism of action of antineoplastic agents can be due to two distinct processes of necrosis or apoptosis in the cells. Cell death by necrosis is a more passive form of cell death that is characterize by organelle and cell swelling, loss of membrane integrity, rupture of the plasma membrane and cell lysis (Dive et al., 1992). Necrosis is often associated with extensive tissue damage and an intense inflammatory response (Alison et al., 1995). Apoptosis, on the other hand, is an active process that involves the activation of various cell-signaling cascades which results in characteristic morphological and biochemical changes such as chromatin condensation, DNA fragmentation, membrane blebbing, and cell shrinkage (Rieux-Laucat et al., 2003). The cell is eventually broken down into smaller membrane-bound vesicles termed apoptotic bodies that engulfed by surrounding cells without initiating an inflammatory response (Rieux-Laucat et al., 2005). Specific compounds in the extracts of *Isatis tinctoria* from China have different effects on cell lines, for example, roots containing high values of Indirubin and leaves have indicant and isatan B and C (Wu, 1982; Maugard, 2001). Different sensitivities of cell lines and steps in our experiments could also contribute to a variation of results.

However many modern drugs have their origin in the traditional medicine, plants are cheap and safe in comparison to synthetic compounds, especially endemic medicinal plants which are more available than foreign medicinal plants. Moreover, some additional compounds in plant extracts can prevent the side effects of the synthetic drug.

In conclusion, we observed the anti cancer activity of *I. campylocarpa* plant against different tumor cell lines. More in vitro and in vivo studies need to discover the chemical composition and anti cancer characteristics of the extracts and further more mechanistic work is essential to prove the compounds of the extracts as a one of the specific cancer drug.

Table 1. IC50 values of *I. campylocarpa* extracts on three examined cell lines

Extract	Cell line	IC50 ($\mu\text{g/ml}$)		
		Jurkat	K562	Fen
Stem alcoholic extract		128.8	>200	97.7
Root alcoholic extract		125.8	123.02	39.8
Leaf alcoholic extract		199.5	125.89	85.11
Stem aqueous extract		>200	>200	107.15
Root aqueous extract		10.2	>200	72.44
Leaf aqueous extract		50.1	>200	154.88
Seedling aqueous extract		>200	>200	173.78
Positive control		<5	<5	<5

**Figure 1.** Effect of different concentrations of *I. campylocarpa* extracts on cells growth. Values represent the mean of three experiments.

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