Cytotoxic effect of *Chelidonium majus* on cancer cell lines

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Abstract

Cancer is a growing health problem coming next to the cardiovascular diseases regarding the morbidity and mortality with some international geographical variation according to its type. The purpose of the present study was to investigate potential *in vitro* cytotoxic effect of the aqueous extract of *Chelidonium majus* on two human cancer cell lines (pelvic: RD and cervical: HeLa). The extract exhibited a time- and concentration-dependent inhibitory effect on both cell lines. The highest significant inhibitory effect of extract recorded after 72 hrs of exposure at highest concentration (1000 µg/ ml), it was achieved 58.4% and 65.1% on RD and HeLa, respectively. The treatment with 500 and 1000 µg/ml of extract caused significant reduction in viability of RD and HeLa cells during all periods of exposure. These results revealed that *C. majus* possessed cytotoxic effect on cancerous cell lines, which makes it has possible role in medical oncology.

التأثير السمي للمستخلص المائي لنبات عروق الصباغين *Chelidonium majus* في بعض الخطوط الخلوية السرطانية

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الخلاصة

يعتبر السرطان مشكلة صحية متنامية تأتي بعد أمراض القلب والشرايين من حيث معدل الأضرار والوفيات مع الاختلاف في التوزيع الجغرافي العالمي اعتماداً على نوع السرطان. إن الغرض من هذه الدراسة هو فحص القابلية السمية في الزجاج للمستخلص المائي لنبات عروق الصباغين (*Chelidonium majus*) ضد الخطين الخلويين لسرطان العضلات المخططة البشري (RD) وسرطان عنق الرحم (HeLa). لقد أظهر المستخلص تأثيراً تثبيطياً معتمداً على التركيز المستخدم منه ومدة التعريض تجاه كلا الخطين الخلويين، فظهر التأثير التثبيطي المعنوي الأعلى عندما عوملت الخلايا بالتركيز 1000 مايكروغرام/ مل لمدة 72 ساعة، حيث وصل 58.4% و65.1% لخلايا RD وHeLa على الترتيب. وأبدى المستخلص بالتركيزين 500 و1000 مايكروغرام/ مل تأثيراً تثبيطياً معنوياً في كلا خطي الخلايا لجميع مدد التعريض المستخدمة. هذه النتائج تُظهر بأن المستخلص يمتلك تأثير سمّي ضد الخطوط الخلوية السرطانية، والذي يجعل له دوراً محتملاً في علاج الأمراض السرطانية.

Introduction

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans well as valuable components of seasoning, beverages, cosmetics, dyes and medicine. Most of this therapy involves the use of plant extracts or their active components (1). At least 250.000 species of plants do exist, out of which more than one thousand plants have been found to possess significant anticancer properties (2). The National Cancer Institute (NCI) collected about 35.000 plant samples from 20 countries and has screened around 114.000 extracts for anticancer activity (3). *Chelidonium majus* Linn. (greater celandine, swallow-wort, and bai-qu-cai in Chinese) is the only species of the tribe Chelidonieae of the Papaveraceae family. It is rich in various types of isoquinoline alkaloids. The phytochemical and pharmacological properties, including spasmolytic, antiulcer, anti-inflammatory, antibacterial, antiviral, antifungal and antioxidant activity of *C. majus* have been reviewed, which is of great interest for its use in Asian, European and Chinese herbal medicines (4, 5, 6, 7). *C. majus* has been traditionally used for treatment of gastric ulcer, astric cancer, oral infection, liver disease, and general pains. The extracts of *C. majus* are proven to be safe as components of veterinary and human phytopreparations, and of oral-hygiene agents (8). Previous chemical studies of *C. majus* have reported the isolation of isoquinoline alkaloids such as chelidonine, chelerythrine, sanguinarine, berberine, coptisine, and *dl*-stylopine (9). Among them, chelindonine and protopine exhibited anti-tumor activity, and protoberberine showed antibacterial and antiviral activity, while sanguinarine and chelerythrine had anti-inflammatory activity (9). Currently, one key component of a comprehensive preclinical screening and drug development program at the NCI is the cell-line screen (10). In Iraq, many efforts were begun for finding alternative treatment of cancer disease (11). Our study aimed at evaluate the cytotoxic effect of *C. majus* extract against malignant cell lines.

Material and Methods

- **Preparation of the *C. majus* extract:** Distilled water was added to powdered plant leaves in a ratio of (1:5 W/V) with continuous stirring for 3 days at room temperature (18-25°C). The mixture was then filtered through a piece of soft cloth, filter paper and centrifugation to remove all the residual materials. The clear solution of the extract was dried at 45˚C by using oven and kept at 4˚C until use (12).
- **Cell lines:**

- Rhabdomyosarcoma (RD) cell line was kindly provided by Iraqi Center for Cancer and Medical Genetics Research (ICCMGR). This human cell line was derived from a biopsy specimen obtained from a pelvic rhabdomyosarcoma of a 7-year-old Caucasian girl (13).
- A HeLa cell line was kindly provided by ICCMGR. This human cell line was derived from cervical cancer cells taken from Henrietta Lacks, who died from her cancer on October 4, 1951. These cells are treated as cancer cells, as they are descended from a biopsy taken from a visible lesion on the cervix as part of Mrs. Lacks' diagnosis of cancer. A debate still continues on the classification of the cells (14).

Both cell lines were cultured in RPMI 1640 medium (Bio Whittaker) containing 20% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin.

- **Assay for cytotoxic activity:** Cell cultures in the micro-titration plate were exposed to a range of plant extract concentrations during the log phase of growth and the effect was determined after recovery time. The following protocol (15) was

performed on *C. majus* extract: After trypsinization, cell suspension seed in a micro-titration plates at 50000 cells/ml RPMI-1640 growth medium with serum 5% used for seeding. Plates then incubated for 24 hours. By using maintenance medium (free of serum), two-fold serial dilutions were prepared starting from 1000 µg/ml ending with 62.5 µg/ml. After incubation for 24hrs, and when the cells are in the exponential phase, remove the medium and add extract serially across the plates using replicates of four wells per concentration. Add fresh growth medium to the control wells. Only 20 µl of each concentration added for each well. 20µl of maintenance medium added to each well of control group. The time of exposure were 24, 48 and 72 hours. The plates sealed with self adhesive film then returned to the incubator at 36.8 ºC. After the end of the exposure period, the medium decanted off and cells in the wells gently washed by adding and removing 0.1 ml sterile phosphate buffer saline two times. Finally, 50µl of 0.01% crystal violet dye added. After 20 min. the stain was washed gently with tab water for three times. The plate was left until become dry. The optical density of each well was read by using a micro-ELISA reader at 495 nm transmitting wavelength (15, 16). The percentage of inhibition was calculated according to the following equation (17):

Inhibition % = 100- [(optical density of test wells/optical density of control wells)] × 100

- **Statistical analysis:** Analysis of variance (ANOVA) and the least significant difference (LSD) were used for the statistical analysis. These calculations were carried out according to program SPSS version/10.

Results

The cytotoxic effect of two-fold of *C. majus* extract during 24, 48 and 72 hrs of exposure on RD and HeLa cell lines are shown in Table (1) . The extract showed a time- and concentration-dependent effect on viability of both cell lines. Viability decreased with time reaching its lowest after 72 hrs of treating with all concentrations used. The treatment with 1000 µg/ml after 72 hrs caused highest reduction in viability of RD and HeLa cells thus reaching 41.6% and 34.9% (percentage of inhibition 58.4% and 65.1%), respectively (Fig. 1, 2). Interestingly, treatment with 500 and 1000 µg/ml of extract caused significant reduction in viability of RD and HeLa cells during all periods of exposure. At 72 hrs duration particularly, the significant reduction of RD viability observed with 125, 250, 500 and 1000 µg/ml concentrations (Table 1). Compared with inhibition of RD cells, HeLa inhibition by the extract was higher. This reflected the sensitivity of HeLa cells compared with RD cells.

Table (1) A comparison of growth inhibition percentage of RD and HeLa cells, by aqueous extract of *Chelidonium majus* during three periods of exposure

Concentration (µg/ml)	RD			HeLa		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
62.5	1.4	1.2	10.4	0.5	2.6	24.5
125	---	3.6	37.4*	0.4	8.1	25.1
250	0.8	3.2	39.7*	4.6	18.4	24.8
500	29.3*	28.7*	51.2*	28.7*	46.0*	51.8*
1000	44.2*	48.3*	58.4*	45.5*	61.1*	65.1*

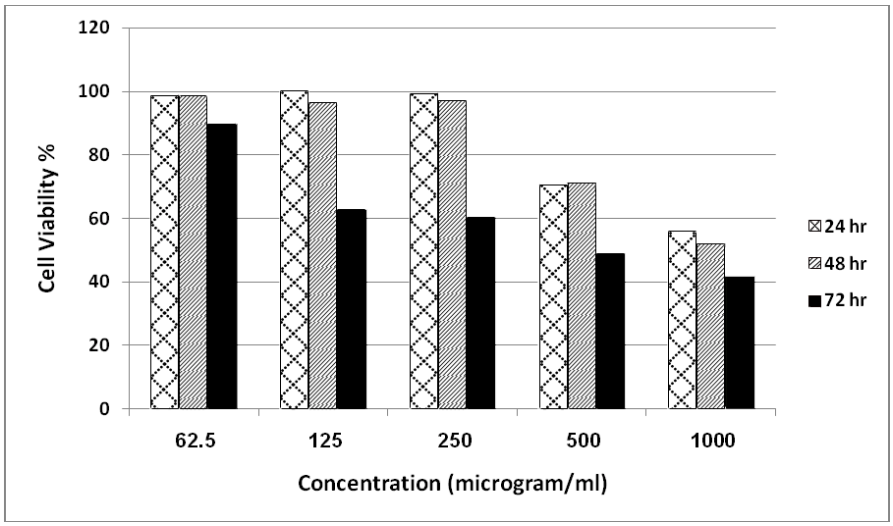

Fig. (1) Effect of aqueous extract of *Chelidonium majus* on viability of RD cells

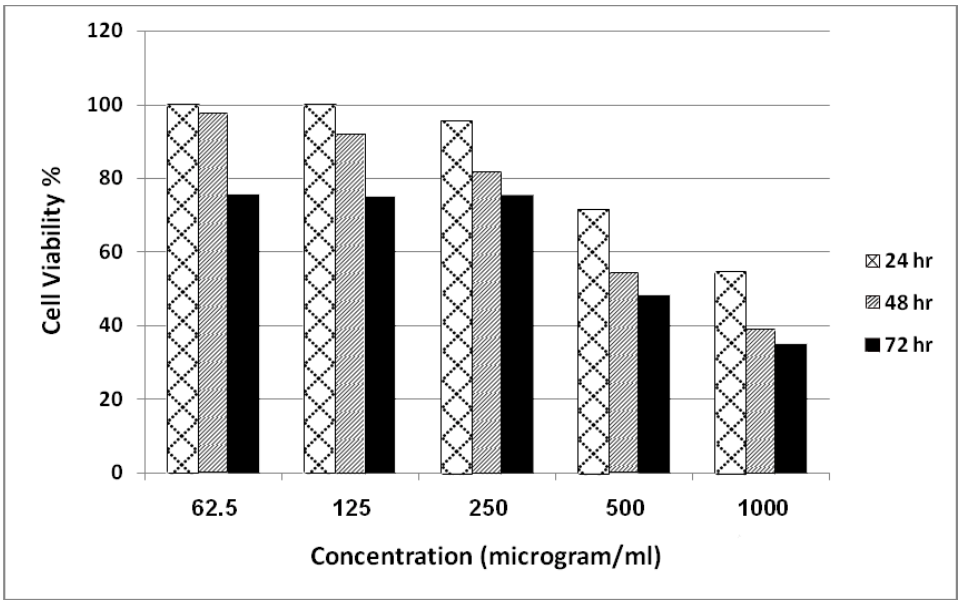

Fig. (2) Effect of aqueous extract of *Chelidonium majus* on viability of HeLa cells

Discussion

Cancer chemotherapy with strategies using foods and medicinal herbs has been regarded as one of the most visible fields for cancer control (18). *Chelidonium majus* L. has a long history as being useful for the treatment of many diseases over the world. The plant contains, as major secondary metabolites, isoquinoline alkaloids, such as sanguinarine, chelidonine, chelerythrine, berberine and coptisine. Other compounds structurally unrelated to the alkaloids have been isolated from the aerial parts: several flavonoids and phenolic acids.(1). In this study, crude extract was used because of the biological activity, if proven to exist, might be lost during the process of purification from the crude extract (19). Furthermore, it was proposed that the additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent antioxidant and anticancer activities, and that the benefit of a diet rich in fruit and vegetables is attributed to the complex mixture of phytochemicals present in whole foods (20). Water soluble compounds may present in the aqueous extracts. Kamuhabwa

and his colleagues reported that polar compounds, responsible for anticancer activity are water soluble (21). The classical method for evaluating the effect of deleterious treatments on cell is based on proportion of inhibition (17), which indicate the rate of inhibition of cell growth (22) or percentage of toxicity (23). These parameters are used in present study for evaluation of cytotoxic effect of *C. majus* extract. In this study, *C. majus* aqueous extract showed a time- and concentration-dependent effect on viability of both cell lines. The same effect appeared when Jagetia and Rao studied the effect of *Tinospora cordifolia* extracts on HeLa cells (24). Also The aqueous extract of *Moringa oleifera* showed good cytotoxicity on HeLa cells which was concentration dependent (25).Yang and his colleagues reported that the saponin ginsenoside, isolated from *Panax notoginseng*, inhibits the cell growth of HeLa cells in a concentration- and time-dependent manner, with an IC(50) value of 150.5+/-0.8 mcirog/ml after 48 hr of incubation (26). Compared with inhibition of RD cell, HeLa inhibition by *C. majus* aqueous extract was higher. This reflected the resistance of RD cells compared with HeLa cells. Harput and his colleagues showed in their study on effects of The water extract of *Moltkia aurea* Boiss that RD cells were more resistant to the extract compared with Hep-2 (human larynx epidermoid carcinoma) and L-20B (transgenic murine L-cells) (27). Aqueous extract of *C. majus* at higher concentrations exert a direct cytotoxic effect on both malignant cell lines. This finding indicate that *C. majus* may be useful as cancer chemotherapeutic agents. The most accepted explanation for the cytotoxic effect of plant extract is the ability of plants to induce the programmed cell death in cancerous cells, as attempt to arrest their proliferation. A number of food items as well as herbal medicine have been reported to produce toxic effects by inducing programmed cell death (28).

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