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Original Article

Cytotoxic effect of *Convolvulus arvensis* extracts on human cancerous cell line

H. Sadeghi-aliabadi[1,2,*], N. Ghasemi[3] and M. Kohi[2]

[1]*Department of Medicinal Chemistry, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R.Iran.*
[2]*Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R.Iran.*
[3]*Department of Pharmacognosy, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R.Iran.*

Abstract

It has been reported that several members of Convolvulaceae family such as *Convolvulus arvensis* possess cytotoxic activity on some tumor cell lines. Proteoglycan mixture, the water extract of *C. arvensis* with high molecular weight, has potent anti-angiogenesis and stimulating effects. In this study, arial parts of *C. arvensis* were collected and identified, and the cytotoxic effects of chloroform, ethylacetate and hydroalcoholic extracts on human tumor cell line (Hela) were determined. Different concentrations of the extracts were added to the cultured cells and incubated for 72 h. Cell survival was evaluated using MTT assay. The chloroform extract of *C. arvensis* at tested concentrations showed a comparable cytotoxic effect to taxol against Hela cell (15 vs. 12 µg/ml). In conclusion, the higher cytotoxic activity seen with chloroform extract may be related to the presence of lipophilic glycosides as non-polar compounds extracted by chloroform.

Keywords: *Convolvulus arvensis*; Proteoglycan mixture; Hela; MTT assay

INTRODUCTION

Plant materials represent promising sources of anticancer agents with lower side effect as compared with chemical drugs. A novel proteoglycan mixture (PGM) extract from ubiquitous plant *Convolvulus arvensis* was developed and tested on S-180 fibrosarcoma, heparin-induced angiogenesis in chick embryo, lymphocytoma and tumor cell growth. The results showed that extracts inhibited tumor growth and angiogenesis in chick embryo and improved lymphocyte (1,2).

Previous preliminary studies have revealed that different members of the family of Convolvulaceae possess cytotoxic effects against a number of tumor cells. The potent compound present within methanolic extract of *Ipomoea aquatica,* responsible for its cytotoxic activity, was poly-phenolic compound (3); whereas in other species such as *I. pes-caprae*, *I. stan* and *I. murucoids* lipophilic glycoside compounds were responsible for cytotoxic activity of none polar extracts (4-6).

Literature reviews revealed that no study has been carried out on cytotoxicity of different extracts of *C. arvensis* on Hela cell. Therefore this study was conducted to evaluate the cytotoxic effect of three extracts of forementioned plant on this cell line.

MATERIALS AND METHODS

Plant material

Arial parts of *C. arvensis* were collected from Isfahan University campus in April 2005. It was identified by the Department of Botany, Isfahan University of Medical Sciences (Isfahan, Iran) and stored at -20 ºC before use. Voucher specimen (No. 1520) of the plant was deposited in the herbarium of the School of Pharmacy and Pharmaceutical Sciences, Isfahan

*Corresponding author: H. Sadeghi, PhD
Tel. 0098 311 6688064 and 7922564, Fax. 0098 311 6680011
Email: sadeghi@pharm.mui.ac.ir

University of Medical Sciences (Isfahan, Iran).

Extraction and isolation

One hundred gram of plant materials was used for extraction. Hydroalcoholic (ethanol 70%), chloroform and ethyl acetate extracts were prepared by percolation, reflux and soxhlet, respectively and, extraction times were fixed for 72 h, 1 h and 4 h, respectively.

The extracts were concentrated by rotary evaporator and dried in an oven at 40 ºC. To prepare the stock solutions, 2 mg of each solid residue was dissolved in 140 µl of DMSO and RPMI-1640 was added up to 1 ml. The mixtures were then filtered and sterilized using 0.22 µ microfilters and kept frozen. Serial dilutions (100, 75, 37.5, 18.75 and 9.375 µg/ml) were freshly prepared from stock solution before use.

Cell line

Hela cell line was purchased from Pasture Institute (Tehran, Iran). Cells were grown in RPMI-1640 [each 500 ml of RPMI-1640 was supplemented with 10% of fetal calf serum, 5 ml of penicillin/streptomycin (50 IU/ml and 50 µg/ml respectively), 5 ml of sodium pyruvate (1 mM), $NaHCO_3$ (1 g) and 5 ml of L-glutamine (2 mM)]. The final medium was then sterilized using 0.22 µ microfilters and stored at 4 ºC before use.

MTT-based cytotoxicity assay

Cytotoxic effect of the extracts against Hela cells was determined by a rapid colorimetric assay, using 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and compared with taxol as a positive control (7).

This assay is based on the metabolic reduction of soluble MTT by mitochondrial enzyme activity of viable cells into an insoluble colored formazan product, which can be measured spectrophotometrically after dissolving in DMSO (8). Briefly 180 µl of cell suspension (3×10^4 cell/ml) were seeded in 96 well microplate and incubated for 24 h (37 ºC, 5% CO_2 air humidified). In the next day 20 µl of prepared concentrations of each extract was added in triplicate. The negative control contained no extract or taxol and the positive control contained 20 µl of taxol (2 µM). Microplate containing cells and extracts were incubated for another 72 h in the same condition.

To evaluate cell survival, 20 µl of MTT solution (5 mg/ml in phosphate buffer solution) was added to each well and incubated for 3 h. 180 µl of the old medium containing MTT was then gently replaced by 150 µl DMSO and pipetted to dissolve any formazan crystals formed. Absorbance was then determined at 540 nm by an ELISA plate reader. Each extract concentration was assayed in 3 wells and repeated 3 times. Standard curve (absorbance against number of cells) for the cell line was also plotted.

Cell survival percentage was calculated based on the following formula. Percentage of cell survival in the negative control was assumed as 100.

$$\%\ Cell\quad Servival\quad = \frac{At\ -\ Ab}{Ac\ -\ Ab}\times 100$$

A_t: Absorbance of tested concentration,
A_b: Absorbance of blank,
A_c: Absorbance of negative control

Statistical analysis

SPSS was used to perform statistical tests. Analysis of variance was used to distinguish the difference among groups. Significance was assumed at 5% level. For detecting the point of difference the post hoc test was used.

RESULTS

According to Fig. 1, chloroform extract showed the highest cytotoxic effect among the extracts (IC_{50} = 15 µg/ml) whereas ethyl acetate and hydroalcoholic extracts were less cytotoxic against Hela cells (IC_{50} = 25 and 65 µg/ml, respectively). To determine the IC_{50}s, a concentration of 20 µg/ml of dried residue of all extracts were used and their cytotoxic effects were evaluated (Table 1). Using NCI criteria only crude extracts with a concentration of $\leq$20 µg/ml are useful for further evaluation; therefore chloroform extract could be a good candidate for future studies.

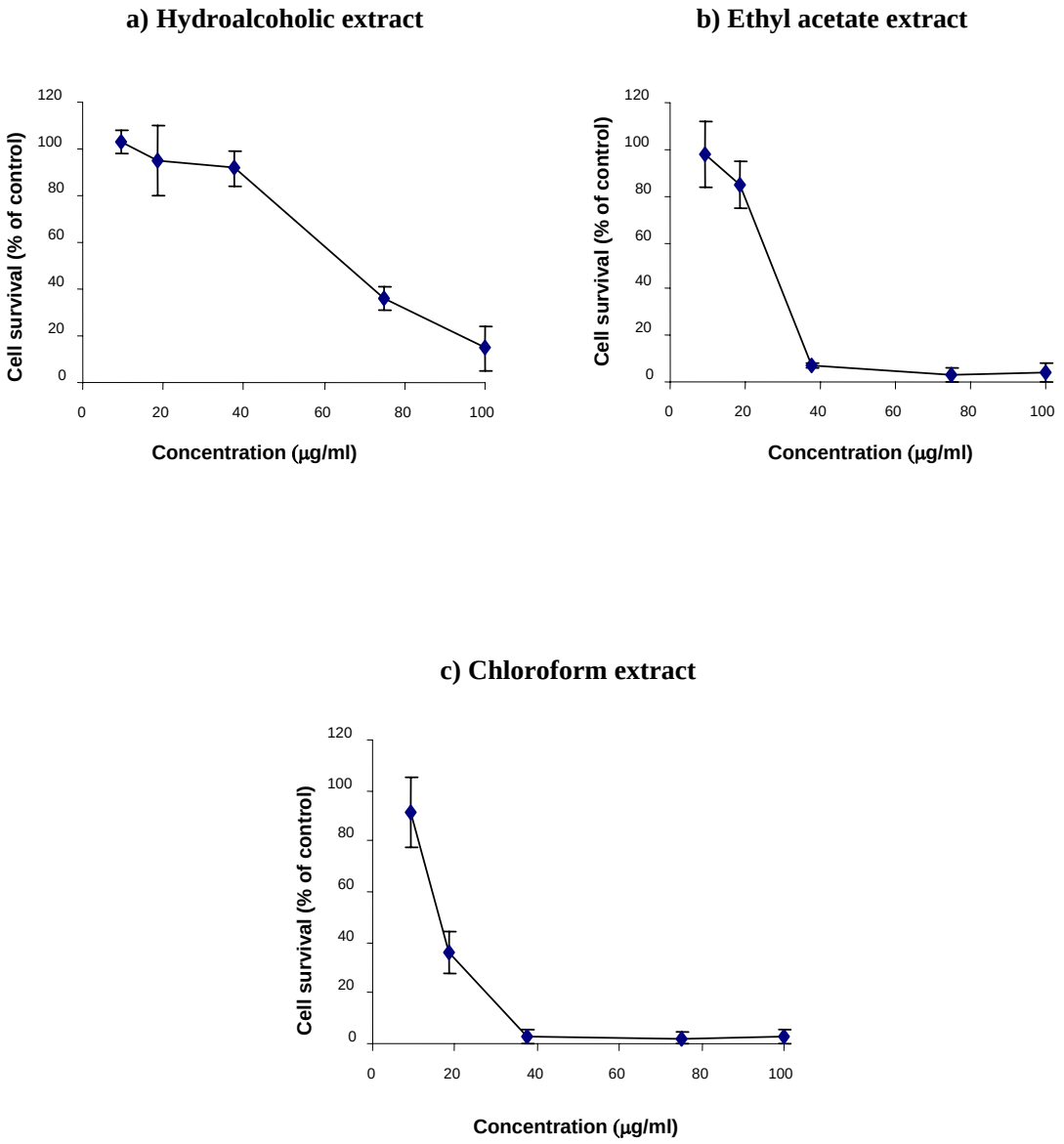

Fig. 1. Dose response curves for Hela cells (3×10^4 cell/ml) survival following continuous 72 h exposure to hydroalcoholic (a); ethyl acetate (b) and chloroform (c) extracts of *C. arvensis*. Results represent the Mean ± SD, (n=6).

DISCUSSION

Toxicity of *C. arvensis* in mice had been investigated many years ago (9). This effect has been related to several tropine alkaloids, including pseudotropine. On the other hand, some studies indicated that PGM presented in water extract of this plant is able to inhibit tumor growth and angiogenesis in some tumor cells (1,2). In these studies *C. arvensis* extracted in boiling water (containing proteins and polysaccharides) inhibited tumor growth in a dose-dependent fashion. They showed that at the highest dose tested, 200 mg/kg/day, tumor growth was inhibited by 70%. Polyphenolic compounds as components of methanolic extract of *I. aquatica* (convolvulaceae) are shown to be cytotoxic on some tumor cells (3). In our studies primary phytochemical studies showed the presence of polyphenolic compounds such as flavonoid and tannins in this plant (10). These components and also PGM are polar and seem to be more extracted by hydroalcoholic

Table 1. IC_{50} values of 3 different extracts of *C. arvensis* against Hela cells

No.	Extraction solvents*	IC_{50} (µg/ml)
1	chloroform	15
2	ethyl acetate	25
3	hydroalcoholic	65

*Extractions were performed as mentioned in experimental section. 20 µl of dried crude extracts with a concentration of 20 µg/ml were tested against Hela cells and IC_{50} were determined, using dose-response curves in Fig. 1.

solvents than chloroform; but the results showed less effect with polar extract. The higher effect of chloroform extract indicated that some other compounds extracted in non-polar solvent are responsible for its effects. Pereda-Miranda and co-workers in their study showed that cytotoxic effect of hexane extract of *I. pes-caprae* is related to some lipophilic glycoside compounds (6). In other study Leon et al. showed effect of chloroform extract of *I. murucoides* on tumor cell related to 5 types of lipophilic glycoside compound (5). These types of compounds are responsible for cytotoxic effect of *I. bahiensis* and *C. arborescen* on tumor cell lines (11,12). Regarding to this point non-polar compounds that are responsible for the cytotoxic effect may be lipophilic glycoside. This point needs to be more explored in future studies.

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