

Original Research Article

Cytotoxic Effect of Ethanol Extract of *Convolvulus arvensis* L (Convolvulaceae) on Lymphoblastic Leukemia Jurkat Cells

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Abstract

Purpose: To evaluate the cytotoxic effect of ethanol extract of aerial parts of *Convolvulus arvensis* against lymphoblastic leukemia, Jurkat cells.

Methods: The aerial parts of *C. arvensis* were collected, identified, powdered and soaked in ethanol. The extract was filtered and evaporated, and the residue assessed for cytotoxic activity in Jurkat cell line. The cells were exposed to different concentrations (10, 25, 50, 75 and 100 µg/mL) of the extract to determine cell viability, cell proliferation, apoptosis using Trypan blue exclusion assay, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay and fluorescent activated cell sorter (FACS) analysis, respectively.

Results: Trypan blue exclusion assay and MTS assay results indicate that the ethanol extract decreased the number of living cells in a concentration-dependent fashion. The results of FACS analysis showed that the lowest concentration of the extract (10 µg/mL) was most effective for the induction of apoptosis as it induced maximum apoptosis (85.34 %) and the highest concentration (100 µg/mL) was less effective as it induced less apoptosis (53.70 %) in Jurkat cells ($p < 0.05$).

Conclusion: The ethanol extract of *C. arvensis* has significant cytotoxic activity against the selected cancer cell line. Furthermore, apoptotic effect was more prominent at lower doses and necrosis at higher doses of the extract.

Keywords: *Convolvulus arvensis*; (MTS) 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay; Trypan blue exclusion assay; Apoptosis, Necrosis

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INTRODUCTION

Convolvulus arvensis belongs to the family Convolvulaceae and commonly known as field bindweed [1]. It is extensively cultivated in the United States, North America, Canada and California as well as in the vicinity of Pakistan especially in canal irrigated wheat fields like Sahiwal, Qasoor and Gujrat, etc. Traditional

medicine system had used this plant from the 1730s because it had great potential as a therapeutic agent [2]. Extracts of Convolvulaceae family ironically known as "the weeds of cancer," hold great potential against cancer. Previous studies revealed that other members of this family possessed cytotoxic activity against a number of tumor cells and a new research has shown that *C. arvensis* is a promising candidate

as a useful, safe and nontoxic chemotherapeutic agent [1]. Aerial parts of *C. arvensis* are used as a laxative, anti-spasmodic, in wound healing and anti-hemorrhagic activities [3]. Other reported activities include antioxidant activity against chromium (III) and chromium (VI) stress, anti-tumor and anti-angiogenesis activities and at high doses shows toxicity such as hepatic necrosis and gastritis with ulceration or erosions in mice and angiogenesis effect [3,4]. Its immune-stimulant effect has also been reported [5].

Phytochemical analysis had indicated the occurrence of caffeic acid, lipids, δ -amino levulinic acid and alkaloids including cuscohygrine and calystegines [1,3]. It also has polyphenolic compounds including flavonoids and tannins in its aerial parts [6] as well as thirteen saponins [7]. Its leaves contain flavonoids and glycosides like Kaempferol 3-mono- glycosides and Quercetin 3-mono or diglycosides [8]. Against a variety of diseases all aerial parts of *C. arvensis* have been ethno medicinally used as therapeutic agents. This study evaluated the cytotoxic activity of ethanolic extract of *C. arvensis* on lymphoblastic leukemia Jurkat cell line.

EXPERIMENTAL

Plant material

The assemblage of aerial parts of *C. arvensis* were done from the fields of Punjab Pakistan from January to April 2011 and identified by Dr. Mubashar Niaz, Department of Botany, GC University Faisalabad Pakistan. A voucher specimen with no. 010410 was kept in the Herbarium, College of Pharmacy, GC University, Faisalabad, Pakistan. The parts of *C. arvensis* were washed, shade-dried, powdered and stored at room temperature.

Extraction and isolation

The powdered plant was soaked for 3 to 5 days in ethanol (polar solvent) and shook occasionally. The entire material was filtered and using rotary evaporator, the filtrate was evaporated to get a solidified mass which was stored at 4 °C. After filtration stock solution was prepared by adding 10 mg of extract in 1 mL solution containing 500 μ L ethanol and 500 μ L water. The stock solution was used for preparing fresh serial dilutions having concentrations of 10, 25, 50, 75 and 100 μ g/mL for evaluation of the cytotoxic activity of *C. arvensis* ethanol extract on the selected cell line.

Cell line and culture conditions

After obtaining the cell, the humidified incubator was used for maintaining Jurkat cell line at 37 °C with 5 % CO₂. RPMI-1640 was used for culturing the cells with 10 % (v/v) fetal bovine serum (FBS) (Biowhitaker, Lonza, Belgium), L-glutamine (2 mm), penicillin (100 units/mL) and streptomycin (100 μ g/mL, Sigma, St Louis, MO, USA).

Trypan blue exclusion assay

Trypan blue exclusion assay was used to study the effect of ethanolic extract of *C. arvensis* on viability of Jurkat cell line. At a density of 10⁵ cells/well, these cells were cultivated for 24 h in 6-well plates followed by the addition of either the vehicle (ethanol 0.03%) or different concentrations of the extract. After 24 hours the cells were collected and resuspended in 0.4% trypan blue (Sigma–Aldrich, St-Quentin Fallavier, France). Haemocytometer was used to count the number of viable cells.

MTS assay

MTS assay was used to study the effect of cell proliferation of ethanolic extract of *C. arvensis* on Jurkat cell line. Jurkat cells (2 \times 10⁴) were incubated in complete RPMI-1640 medium in the presence of vehicle for the extract (ethanol 0.03%) or different concentrations of the extract (10, 25, 50, 75 and 100 μ g/mL) for 24 h in triplicate in 96-well plates. After 24 h, 20 μ L of MTS reagent (Aqueous One_Reagent, Promega, United States of America) was added to each well and incubated the plate for 2 h. Thereafter absorbance was measured at 490 nm using cell multiwell ELISA plate reader. Viable cell percentage was calculated as a ratio of the OD value of each sample to the OD value of the vehicle.

Apoptosis analysis

Annexin V-FITC apoptosis kit (BD Pharmingen, USA) was used to calculate early and late apoptosis. Phosphatidylserine, present in the membranes of apoptotic cells was strongly attracted by Annexin V. Jurkat cells were incubated for 24 h with either vehicle (ethanol, 0.03 %) or ethanolic extract, resuspended in binding buffer and then washed. Solution of 5 μ L of V-FITC Annexin and 10 μ L propidium iodide with final concentration of 50 μ g/mL was added to every sample and incubated in the dark for 20 min. Then FACS analysis was executed on the Jurkat cells. Dot plots were used to record and represent at least 10,000 events.

Statistical analysis

The data were expressed as means \pm SEM from at least three experimental readings. One-way ANOVA technique was used for statistical analysis, and the significance level was $p < 0.05$.

RESULTS

Convolvulus arvensis ethanolic extract reduced cell viability of Jurkat cells

The effect of *C. arvensis* extract on cell viability of Jurkat cells was assessed using the trypan blue exclusion assay. The extract significantly decreased the cell viability of Jurkat cells after one day and up to 8 days of treatment in a concentration dependent manner with a maximum inhibitory effect observed at 100 $\mu\text{g/mL}$ as shown in figure 1. Cell viability determination indicates that the extract at various concentration 10, 25, 50, 75 and 100 $\mu\text{g/mL}$ reduced cell viability by 77, 73, 70, 67.6 and 45.6 %, respectively, compared with the control group with 97 % viability.

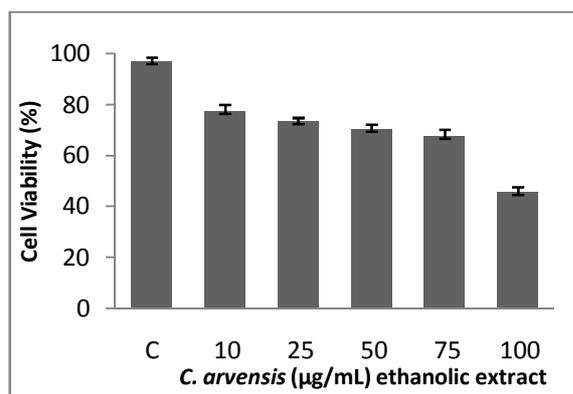


Figure 1: Reduction in cell viability of Jurkat cells was concentration-dependent. Cells were incubated with either vehicle (0.03% ethanol) or extract for 24 h. Viable cells were determined using trypan blue exclusion assay and values were shown as means \pm SEM; $n = 3$, $*p < 0.05$.

C. arvensis ethanol extract reduced cell proliferation of Jurkat cells

The cytotoxic effect of the extract was further assessed using the MTS assay for cell proliferation. This extract significantly decreased the percentage of viable cells in a concentration-dependent manner. For example, in the control group cell proliferation was 97 % but at 100 $\mu\text{g/mL}$ of extract the cell proliferation rate was reduced to 50.3 % as shown in figure 2. These results show that as the concentration of extract

increased, the percentage of viable cells significantly decreased ($p < 0.05$).

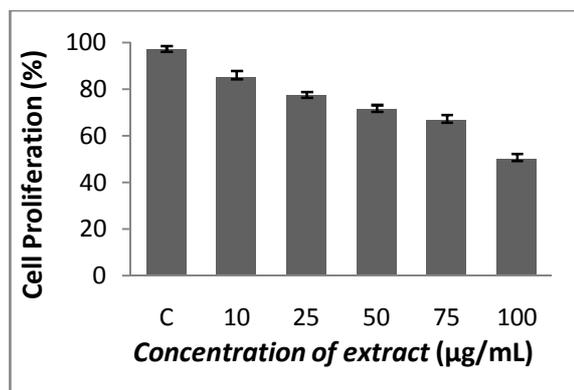


Figure 2: Effect of *C. arvensis* ethanol extract on cell proliferation of Jurkat cells. Values are mean \pm SEM ($n = 3$, $*p < 0.05$).

C. arvensis extract aggravates apoptosis in Jurkat cell line

The extract provoked apoptosis in Jurkat cells. The results of FACS analysis show that *C. arvensis* ethanol extract at concentrations of 10, 25, 50, 75 and 100 $\mu\text{g/mL}$ induced apoptosis 82.6, 68.3, 38, 43.70 and 45.2 %, respectively (Figures 3 and 4). The lowest concentration of the extract (10 $\mu\text{g/mL}$) induced maximum apoptosis (82.20 %) whereas the next higher concentrations (25, 50, 75 and 100 $\mu\text{g/mL}$) were less effective in inducing apoptosis in Jurkat cells.

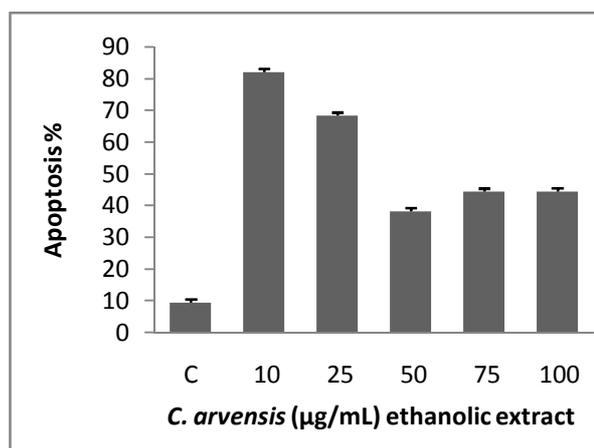


Figure 4: *C. arvensis* induced apoptosis in Jurkat cells. Cells were treated with either vehicle (0.03% ethanol) or *C. arvensis* extract for 24 h. Then they were processed for Annexin V-FITC/propidium iodide double staining and finally subjected to FACS analysis. Corresponding cumulative data. Values are mean \pm SEM; $n = 3$, $*p < 0.05$

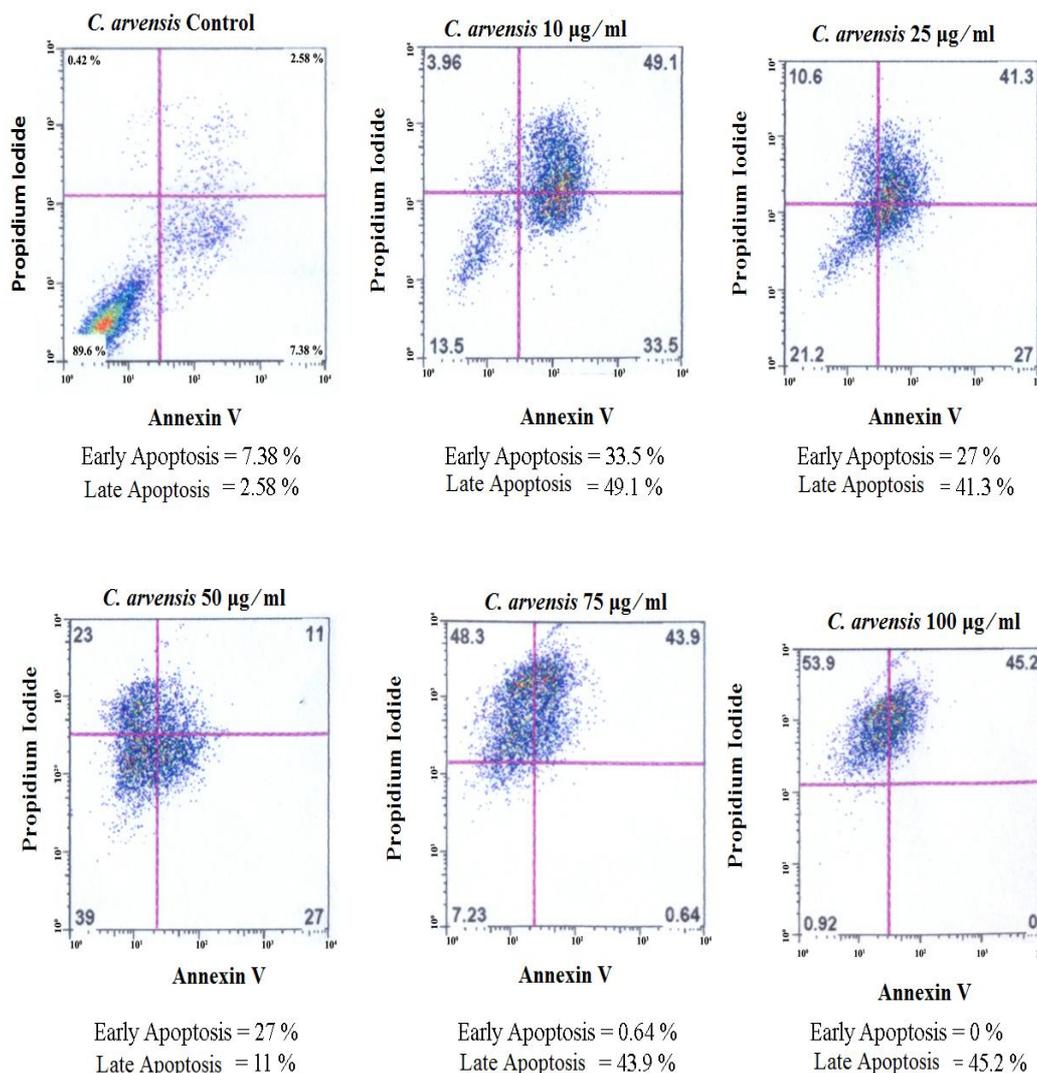


Figure 3: Representative flow cytometry analysis of cells. Each histogram was divided into four quadrants. The cells shown in lower left quadrant were alive and negative for both Annexin V-FITC and propidium iodide. The cells shown in lower right quadrant were in early stage of apoptosis and positive for Annexin-V, The cells shown in upper right were in late stage of apoptosis and positive for both Annexin V-FITC and propidium iodide. The cells shown in upper left quadrant were dead and positive for propidium iodide

DISCUSSION

Now a days, many drugs in-use have become resistant and there is a dire need for the search and development of new drugs [9,10]. Natural products are being considered a good source of new therapeutic agents [11,12]. *C. arvensis* has previously been shown to be cytotoxic against human tumor cell line (Hela) by using MTT assay [13]. In the present study, we have observed the cytotoxic effect of ethanolic extract of *C. arvensis* against Jurkat cells, a human lymphoblastic leukemia cell line, by using Trypan blue, MTS assay and FACS analysis. It was shown from the trypan blue exclusion assay that ethanolic extract of *C. arvensis* decreased cell viability by increasing concentration of the extract. Anti-proliferative activity of *C. arvensis* was

determined by performing the MTS assay. It was shown that synchronized decline in cell proliferation occurred as the concentration of extract increased from 10 to 100 µg/ml. Apoptosis induction was evaluated by FACS analysis. The rate of apoptosis induction by *C. arvensis* ethanolic extract was found maximum (82.2%) at the lowest dose (10 µg/mL). As dose of the extract was increased, apoptotic rate decreased. At the higher doses (50, 75 and 100 µg/mL) apoptosis induction in Jurkat cells was significantly reduced ($p < 0.05$). Thus, 10 µg/ml may be considered the most effective dose for the induction of apoptosis in Jurkat cells. Doses higher than 10 µg/ml decreased the rate of apoptosis induction but at the same time % of necrotic cells or cell debris production increased

as dose of the extract was increased from 10 µg/ml (0.42%) to 100 µg/ml (53.9 %).

Flavonoids and tannins have been proved to be cytotoxic [14] and saponins as anti-cancer agents [15]. Previous studies showed that flavonoids, tannins and saponins were present in ethanolic extract of *Convolvulus arvensis* [16-18]. Therefore, cytotoxic potential of ethanolic extract of *Convolvulus arvensis* may be due to the presence of these compounds.

CONCLUSION

The ethanol extract of *C. arvensis* possesses excellent antiproliferative and proapoptotic activity against Jurkat cells with 10 µg/mL of the extract inducing maximum apoptosis against the cells.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

MS and VS were involved in the study design, acquisition of data and final revision of the manuscript. MS and KH participated in the analysis and interpretation of data and drafted the manuscript. MIQ, BA, MA, US and FN participated in the acquisition and interpretation of data and in the final revision of the manuscript. All authors read and approved the final manuscript.

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