RESEARCH ARTICLE

Cyclamen Exerts Cytotoxicity in Solid Tumor Cell Lines: a Step Toward New Anticancer Agents?

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Abstract

Cyclamen coum is a traditional medicinal plant in the Turkey. Its anticancer properties and whether cyclamen extract induces any cytotoxicity in solid cancer cell lines have not been thoroughly investigated previously. Therefore we examined cytotoxic effects on cervical cells; HeLa and non small cell lung cancer cell, H1299, lines; Cyclamen extract induced cellular death of both HeLa and H1299 cells in a dose dependent manner. We also analyzed the capacity of cyclamen extract to induce apoptosis by the TUNEL method. Here, for the first time we report that the extract of Cyclamen coum, an endemic plant for Turkey, Bulgaria, Georgia and the Middle East can induce cytotoxicity via apoptosis in HeLa and H1299 cells. These results imply that cyclamen extract can be further analyzed to potentially find novel anticancer compounds.

Keywords: Cyclamen coum - HeLa - H1299 - cytotoxicity - apoptosis

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Introduction

Over the past few years, cancer has remained a major cause of death and the number of individuals affected with cancer is continuing to expand (Chaoki et al., 2010). Hence, a major portion of the current pharmacological research is devoted to anticancer drug design customized to fit new molecular targets (Xia et al., 2004). Due to enormous propensity of plants, which synthesize a variety of structurally diverse bioactive compounds, the plant kingdom is a potential source of chemical constituents with antitumor and cytotoxic activities (Kim et al., 2005; Chin et al., 2006). The rich and diverse plant sources of Turkey are likely to provide effective anticancer agents (Ozmen et al., 2009; 2010).

Cyclamen coum is found in the mountains and coastal areas that border the southern and eastern Black Sea coasts from Bulgaria in the west through Georgia and the Crimea in the east. It is also found in the Elburz Mountains of northern Iran. It extends from the Hatay in southern Turkey, down the eastern Mediterranean coast through Syria and the Lebanon into Israel. It grows in shady places in coniferous and broadleafed woodland and scrub, sometimes growing amongst tree roots and rocks (Yesson et al., 2006; Grey-Wilson et al., 2003).

Although this plant is used for recreational purposes, it's medicinal properties remain unexplored. There is a little knowledge on the anti-tumor potential of Cyclamen plant (Indop et al., 2006; Altunkeyik et al., 2012). There is no data in the literature of the anti-tumor potential of compounds of Cyclamen coum. Therefore, the aim of this study was to investigate the potential antitumor effects of Cyclamen coum on Non Small cell lung cancer lines; (NSCLC) H1299 and cervical cancer cell lines; HeLa cells.

Materials and Methods

Preparation of the extract

Cyclamen specimens were collected in the vicinity of Antalya province. Agriculture department was consulted to confirm the validity of specimens. The methanol and water extracts were prepared as described in the literature, in 1 to 2 ratio (weight/weight; 15g plant/60g solvent), evaporated to dryness at 65ºC with a rotator evaporator and stored at -20ºC, with no exposure to light until use (Grey-Wilson et al., 2003). Extracts were diluted with PBS (phosphate buffer saline) to obtain different concentrations of extracts in order to be used in the experiments.

Cell lines and culture conditions

HeLa and H1299 cells were cultured in RPMI 1640 (Sigma aldrich, St. Louis, MO) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA) at 37ºC in humidified incubator with 5%CO_2.

Cell proliferation assay

The medium was aspirated when cells grown to about

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90% confluence. Cells were washed with PBS, trypsinized, counted with a hemocytometer and seeded into 96-well plates ($3x10^4$ cell/ml). After 24 h incubation at 37°C in a 5%CO_2 incubator, the medium was removed and cells were treated with plant extracts added in different concentrations (0.1, 10, 100, 500, 1000, 2000, 4000 µg/ml) to the medium of H1299, HeLa cells for 72hrs. Plant extract concentrations were prepared in PRMI1640 medium which included 10% FCS then sterilized with passing 0.2 micron filter. At the end of incubation periods, medium was removed and cytotoxicity was measured by luminometric method using CytotoxGlo kit (Promega). And IC_{50} values were calculated for the plant extract with respect to the previously published methodology (Gultekin et al., 2006).

Terminal transferase dUTP nick end-labeling apoptosis analysis

HeLa and H1299 cells were trypsinized, counted with a hemocytometer, and then seeded into flasks (3×10^4/mL). For the detection of the induction of apoptosis of cyclamen extract treated, HeLa and H1299 cells were treated with IC_{50} values of each cyclamen extracts for 24 h at 37°C in a humidified incubator with 5%CO_2. At the end of the incubation period late apoptotic events were analyzed by terminal transferase dUTP nick end-labeling (TUNEL) analysis using the In Situ Cell Death Detection Kit (Millipore, Billerica, MA, USA). Apoptotic cells were counted under the microscope.

Statistical analysis

All experiments were performed in replicates of two and repeated independently to confirm the results. Significance of the differences in the means was determined using Student's t test and considering $p<0.05$ to be statistically significant.

Results and Discussion

Cancer remains one of the leading causes of death around the world. The treatment of cancer may benefit from the introduction of novel therapies derived from natural products. Natural products have served to provide a basis for many of the pharmaceutical agents in current use in cancer therapy (Bongiovanni et al., 2006). The need to develop more effective and less toxic anticancer drugs has prompted researchers to explore new sources of pharmacologically active compounds. Natural products have long been used to prevent and treat diseases, including cancers, and might be good candidates to develop anticancer drugs (Kinghorn et al., 1999). Plants have substantial potential to discover active anticancer compounds as most chemotherapeutic drugs, like taxol and vincristin, were already isolated from plants (Pezzuto et al., 1997). Therefore, attempts to search of plant-derived active compounds for new anticancer treatments might be promising.

The present study designed to investigate the potential therapeutic abilities of Cyclamen coum extract in cervical (HeLa) and lung (H1299) cancer cell lines.

Anticancer properties of Cyclamen coum have not been properly studied before. This report is the first to show a meaningful cytotoxic effect of the cyclamen extract in a solid cancer cell lines. Development of novel chemotherapeutics from natural sources is still an important concept, because %30 of anticancer agents currently available are derived from natural sources (Pezzuto et al., 1997).

Isolating novel chemotherapeutics from the cyclamen extract may pave the way for a potential new cytotoxic that may prove useful in the treatment of various cancers.

Our group is currently working to develop potential cytotoxic from the cyclamen extract. Plant derived products are excellent sources for the discovery and development of new anticancer agents (Abdulaev et al.,

Table 1. IC_{50} Values of Cyclamen in Solid Tumor Cell Lines

	HeLa	H1299
Cyclamen extracts	8,61 µg/ml	9,52 µg/ml

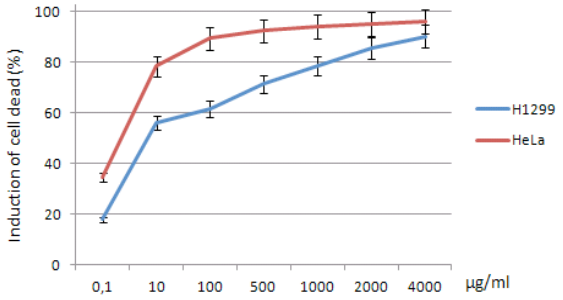

Figure 1. Cyclamen Extracts Have Cytotoxic Effects on HeLa, H1299 Cell Lines. HeLa, H1299 cells were seeded at a density of 3×10^4/mL in 96-well plates. After 24 h, the cells were washed with phosphate-buffered saline, fresh growth medium was added, and then cells were treated with Cyclamen extract in different concentration for 72 h. At the end of the incubation, cell viability was determined in plant extract-treated and untreated control groups by the luminometric method. Data are mean±SD values (n=3). *$p<0.001$ by Student's t test for cytotoxic effects of plant extract compared with the untreated control group

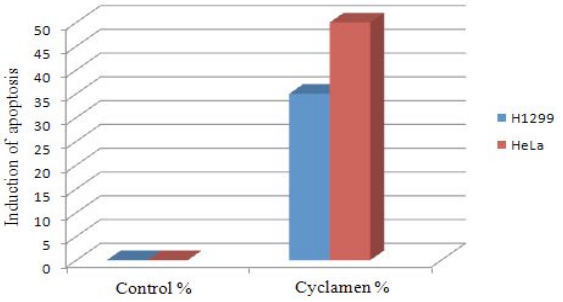

Figure 2. Cyclamen Extract Induces Apoptosis in HeLa and H1299 Cells. HeLa and H1299 cells (3×10^4/mL) were incubated with IC_{50} values of Cyclamen extract for 24 h. After the end of the incubation time cells were washed with phosphate-buffered saline and then assayed by terminal transferase dUTP nick end-labeling analysis using an In Situ Cell Death Detection Kit (Millipore) system to indicate cellular apoptosis. *$p<0.001$ by Student's t test for cytotoxic effects of plant extract compared with the untreated control group

2001). Drug development programs, especially from endemic plant resources still remain to be an important means to discover novel cytotoxic agents.

For the detection of specific cytotoxic activity of both plant extract luminometric analysis was used as described in Materials and Methods. Figure 1 clearly indicates that cyclamen extract has cytotoxic effect on both HeLa and H1299 cells. According to Figure 1 cytotoxic effect of cyclamen extracts on HeLa cell line has stronger than H1299 cell line. We also calculated IC_{50} values of Cyclamen coum in solid tumor cell lines. The cyclamen extract was significantly cytotoxic in HeLa (IC_{50}=8.61 μg/ml) and H1299 cell lines (IC_{50}=9.52 μg/ml) (Table 1). Previous reports also shown that Cyclamen coum extract can induce cellular cytotoxicity (Arslan et al., 2012) our results are good agreement with this previously reports.

To determine whether the induction of cellular death by Cyclamen extract was due to the induction of apoptosis, a TUNEL assay was used. Figure 2 shows that Cyclamen coum extract can induce cellular apoptosis after 24 hours treatment. Even though Cyclamen coum extract have the ability to induce apoptosis on both HeLa and H1299 cell lines, the larger effect on induction of apoptosis was on HeLa cell line.

This study, for the first time reports that Cyclamen coum extract induces moderate cytotoxicity and apoptosis in both cancer cell lines; H1299 and HeLa. The degree of cytotoxicity and apoptosis on cervical cancer cell line HeLa is more strong than on NSCLC H1299 cell line. This selectivity could be due to the sensitivity of the cell line to the active compounds in the extract.

These results implied that cyclamen extracts can have high potential for the development of chemotherapeutic compounds. Obviously, our works show that further work needs towards isolating the main cytotoxic compound of cyclamen, which may be an encouraging step towards development of novel cytotoxics from the extract.

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