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Original Article

Cytotoxic Effect of *Tilia dasystyla* and *Polygonatum orientale Desf* Extracts on AGS and SKOV-3 Cancer Cell Lines

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Abstract

Nowadays medicinal plants have been considered as the complementary medicine in cancer treatment by researchers. Some plants possess chemical compounds which are able to inhibit the growth of cancer cells or even eliminate them through apoptosis or necrosis. In current study anticancer effect of *Tilia dasystyla* and *Polygonatum orientale Desf* extracts on AGS and SKOV-3 cancer cell lines were investigated. The cytotoxic effect of *Tilia dasystyla* and *Polygonatum orientale Desf* extracts on AGS and SKOV-3 has not been reported so far. Cancer cell lines were treated with different concentrations (100-5000 µg/ml) of *T. dasystyla* and *P. orientale Desf* methanol extracts for 24, 48, 72 hours. Cell viability of AGS and SKOV-3 cancer cells were evaluated by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) method. Results of the study indicate that, extracts of *T. dasystyla* and *P. orientale Desf* showed cytotoxic effect on AGS and SKOV-3 cancer cell lines. The lowest IC_{50} value of AGS and SKOV-3 cell lines was about 1.02 ± 0.01 mg /ml and 1.14 ± 0.17 mg / ml respectively. *T. dasystyla* and *P. orientale Desf* extracts showed cytotoxic effect on AGS and SKOV-3 cancer cell lines in time- and dose-dependent manner. Full potential of the extracts, as an option for cancer treatment, is yet to be determined by further studies on animal models and subsequent trials.

Keywords: Anticancer, AGS, Cancer, *Polygonatum orientale Desf,* SKOV-3, *Tilia dasystyla.*

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1. Introduction

Cancer cells are able to invade and metastasize to the surrounding tissues and other organs. Anticancer drugs are supposed to eliminate cancer cells without having side effects on normal cells; however, chemical anticancer drugs have various side effects that pose risks for normal cells. Natural compounds are suitable option for synthesis of

safe anticancer drugs [1]. Gastric cancer is the third leading cause of cancer death and one of the most malignant diseases. This cancer has been seen more often in men and in developing countries. Over 95% of gastric cancer cases are adenocarcinomas. Ovarian cancer is the deadliest cancer in women. Approximately 90% of the malignant tumor of the ovary develops in the surface layer of the ovary, which is called epithelial ovarian cancer [2, 3].

Natural products such as terpenoids and other secondary metabolites have been found in a variety of medicinal plants. These compounds have antioxidant, cytotoxic, anti-mutagenic, anti-inflammatory and anticancer activities, which could prevent cells from destructive effects of free radicals and oxidative damages [4, 5]. Natural compounds in medicinal plants improve the immune system, prevent the spread of cancer cells by inhibition of angiogenesis, enhancement of detoxification and prevention of further increase in toxic substances in the body and neutralization of free radicals that cause DNA mutations. Herbal medicines with antioxidant and anticancer properties could be used as the complementary medicine in cancer prevention and treatment [6, 7].

Tilia is a genus in the family of Tiliaceae. This tree is sprawling in the northern forests of northern hemisphere. . Due to the wide range of bioactive compounds such as hydrocarbons, esters, terpenes, quercetin, kaempferol, phenolic compounds, tannins and scopolytin, Tilia is used as herbal medicine, and recommended for symptoms of colds and anxiety, and recognized by the FDA (Food and drug administration) as a safe product [8, 9]. In a study has been reported that *Tilia amurensis Rupr* showed cytotoxic effect on A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines and Tilia × viridis and Tilia cordata Mill extracts show anticancer properties on lymphoma cancer cells. Also lignan isolated from *T. amurensis* has antitumor and anticancer activities [10-12].

Polygonatum orientale Desf belongs to Asparagaceae family which grows in northern Iran.The rhizome is traditionally used for treatment of kidney stones, healing of internal wounds and gynecological wounds, anti-phlegm and rheumatism and anti-diabetes. In several studies have been reported that *P. odoratum* increases the production of antibodies, and it has anti-inflammatory, antiviral and tumor effects. The anti-depressant, anti-inflammatory, anti-fever, antioxidant and antimicrobial activities of *Polygonatum verticillatum*'s rhizome have been reported [13, 14]. Polysaccharides, saponins, phytohormones, glycosides, flavonoids and alkaloids from Polygonatum's rhizome have been isolated. Polysaccharides of Polygonatum have shown anticancer properties on several cancer cell lines. Pharmacological Studies on several species including *P. odoratum, P. verticillatum, P.* cyrtonema, P.kingianum have been reported [15, 16].

The study aims to investigate of the cytotoxic effect of *P. orientale Desf* and *T. dasystyl*a extracts on AGS and SKOV3 cancer cell lines. The cytotoxic effect of *T. dasystyla*

and *P. orientale Desf* extracts on these cancer cell lines has not been reported so far.

2. Materials and Methods

2.1. Preparation of Plant Extracts

Polygonatum orientale Desf and *Tilia dasystyla* species were purchased in July 2017 from Bagh Firouzeh, Tehran. Identification of species was confirmed by botanist expert at Alzahra University, Tehran, IRAN. Leaves of *T. dasystyla* and rhizome of *P. orientale Desf* were used in this research. 1 gr of dried plants were extracted with 100 ml 80 % aqueous methanol solvent for 60 min at 70 °C temperature in water bath. The extracts was centrifuged for 20 min at 2000 rpm, supernatants were purged to dryness using freeze drying process. Powders was dissolved in PBS (Phosphate-buffered saline) to concentration of 20 mg/ml. 0.22 mm filters were used for sterilization of solution and then solution were diluted with DMEM (Dulbecco's Modified Eagle's Medium) culture medium to concentrations of 100-5000 µg/ml [17].

2.2. Cell Culture

Human gastric cancer cell line (AGS), and human ovarian cancer cell line (SKOV-3) were purchased from Pasteur institute of IRAN. Cells were cultured in DMEM-high glucose containing 10% FBS (Fetal bovine serum) and 1% Pen-Strep incubated in 5% CO2 humidified atmosphere and 37 °C incubator [17].

2.3. Cell Viability

Cell viability of cancer cells were detected by MTT assay. Eight-thousand cells for each well were seeded into 96-well plates for 24h. After 24h cells treated with various concentrations of extracts, and incubated for 24, 48,72h in incubator. Subsequently, 10 ml of MTT (5mg/ml) was added to each well, and then cells were incubated for 4 h at 37 °C. The supernatant of each well was removed, 100 ml of DMSO was added to the wells and plates were kept in 10 minutes in incubator, and then absorbance was measured at a wavelength of 570 nm using a microplate reader (Cytation™Biotek, USA) [17].

The percentage of cell viability was calculated using the formula:

(Mean OD treated well) ÷ (Mean OD control well) × 100 = % Cell viability

IC_{50} values are calculated using Graph Pad Prism 8 software.

2.4. Statistical Analysis

Statistical analysis was performed using ANOVA (Tukey) test by SPSS version 24 program and (P value < 0.05) was regarded as significant. Experiments were performed in triplicate and expressed as the means ± standard deviation.

3. Results and Discussion

Cytotoxic effect of *T. dasystyla* and *P. orientale Desf* extracts at various concentrations in three different times (24, 48, 72 hours) were investigated on AGS and SKOV3 cell lines. Methanol was the most efficient solvent in total antioxidants

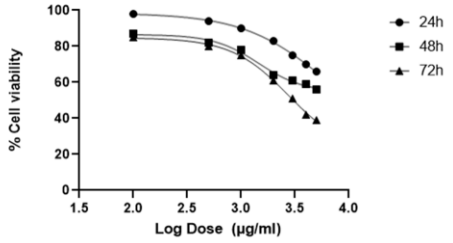

Figure 1. Cytotoxic effect of *P. orientale Desf* extract (100-5000 µg/ml) on cell viability of AGS cell line for 24, 48, 72 h. Experiments were performed in triplicate and expressed as the means ± standard deviation. Values in each column showed significant differences ($P < 0.05$).

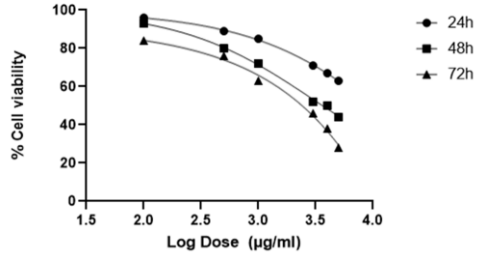

Figure 3. Cytotoxic effect of *P. orientale Desf* extract (100-5000 µg/ml) on cell viability of SKOV-3 cell line for 24, 48, 72 h. Experiments were performed in triplicate and expressed as the means ± standard deviation .Values in each column showed significant differences ($P < 0.05$).

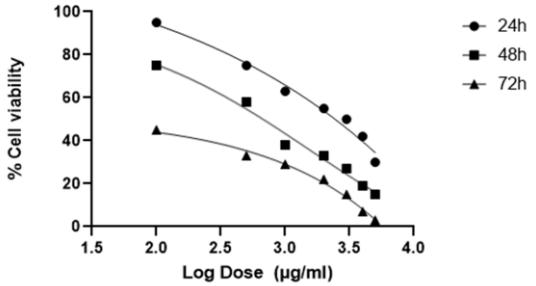

Figure 2. Cytotoxic effect of *T. dasytyla* extract (100-5000 µg/ml) on cell viability of AGS cell line for 24, 48, 72 h. Experiments were performed in triplicate and expressed as the means ± standard deviation .Values in each column showed significant differences ($P < 0.05$).

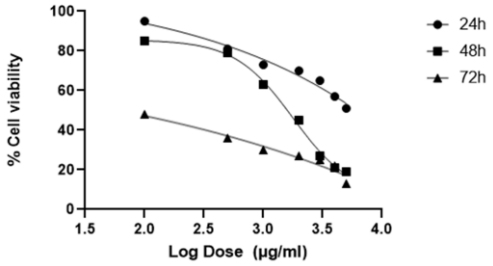

Figure 4. Cytotoxic effect of *T. dasytyla* extract (100-5000 µg/ml) on cell viability of SKOV-3 cell line for 24, 48, 72 h. Experiments were performed in triplicate and expressed as the means ± standard deviation .Values in each column showed significant differences ($P < 0.05$).

extraction, therefor methanol solvent was selected to determine the cytotoxic effect of extracts on AGS and SKOV3 cancer cell lines [16]. According to the data exhibited in Fig. 1-4, the maximum inhibition of cancer cell growth was at 72 h with the concentration of 5 mg/mL for *T. dasystyla* and *P. orientale Desf* methanol extracts causing 97%-61% cell growth inhibition on AGS cell line and 87%-72% on SKOV3 cell line, respectively. According to the IC_{50} values in Table 1, the lowest IC_{50} value that indicates the most effective extract, belonged to *T. dasystyla* extract in both cancer cell lines. The lowest IC_{50} value of AGS and SKOV-3 cell lines were about 1.02 ± 0.01 mg/ml and 1.14 ± 0.17 mg/ml respectively. The highest cytotoxic effect of *T. dasystyla* was on AGS cell line for 72h, at the 5 mg /ml concentration, with 3% cell viability and the highest cytotoxic effect of *P. orientale Desf* was on SKOV-3 cell line for 72h, at the 5 mg /ml concentration with, 27% cell viability.

The results have shown that various concentrations of methanol extract of *T. dasytyla* and *P. orientale Desf* are able to

Table1. Mean IC50 ± SD (mg/ml) values of SKOV-3 and AGS cell lines treated with different concentrations *T. dasystyla* and *P. orientale Desf* extracts (0.1-5 mg/ml) for 24, 48, 72 h.

		IC50 ± SD (mg/ml)		
Extracts	**Cell lines**	**24h**	**48h**	**72h**
T. dasystyla	**SKOV-3**	4.89 ± 0.12	2.12 ± 0.01	1.14 ± 0.17
	AGS	2.95 ± 0.14	1.15 ± 0.02	1.02 ± 0.01
P. orientale Desf	**SKOV-3**	7.19 ± 0.04	3.94 ± 0.08	2.57 ± 0.05
	AGS	7.15 ± 0.16	5.27 ± 0.12	3.43 ± 0.11

inhibit the growth of gastric and ovarian cancer cells with *in vitro* condition. Moreover, the extracts showed cytotoxic effect in time- and dose-dependent manner on AGS and SKOV-3 cancer cell lines. The maximum inhibition of AGS and SKOV-3 cancer cell growth was for 72 h with the concentration of 5 mg/mL of *T. dasystyla* extract, with the percentage of 97% and 87% cell growth inhibition respectively.

The plants may possess diverse groups of components such as flavonoids, phenols, as well as natural antioxidants that might be cytotoxic to cancer cells and not harmful to normal cells [10]. Terpenes, including phenolic and flavonoid compounds are in the non-polar hydrocarbon group; they dissolve better in non-polar solvents such as methanol rather than water, which is a polar solvent. Hanachi *et al*. (2018) has shown that, *T. dasytyla* and *P. orientale Desf* extracted with methanol solvent had higher antioxidant properties than water [16]. In current study methanol solvent were selected and solvent were completely removed after extraction, remained powder were dissolved in PBS to investigate the cytotoxic effect of extracts on AGS and SKOV-3 cancer cell lines.

Based on the results we herein obtained, *T. dasystyla* with a higher cytotoxicity was found to inhibit cell growth more significantly. *T. dasystyla* extract showed the highest cyto toxic effect on AGS cancer cells, for 72h, with the IC_{50} value of 1.02 ± 0.01 mg/ml, and *P. orientale Desf* extract showed the highest cytotoxic effect on SKOV-3 cancer cells, for 72 h, with the IC_{50} value of 2.57 ± 0.05 mg/ml.

There are some studies regarding the cytotoxic effect of the Polygonatum and Tilia genus and on various cancer cell lines have been conducted. Rafi *et al*. (2007) have identified a structure specific homoisoflavone from *Polygonatum odoratum* root which induces Bcl-2 phosphorylation, thereby causing apoptosis breast cancer cells [18]. *Polygonatum cyrtonema* lectin (PCL) , has been reported to show remarkable anti-proliferative and apoptosis-inducing activities in murine fibrosarcoma L929 cells and inhibit the growth of human melanoma A375 cancer cells through a mitochondria-mediated ROS-p38-p53 pathway [19, 20].

The aqueous, dichloromethane and ethanol extracts of *Tilia cordata Mill* flowers showed anti proliferative activity on BW 5147 lymphoma tumor cells and *T. americana var. mexicana* induce remarkable antitumor activity for K-562 leukemia cell line [21, 12].

The methanol extract from the trunk of *Tilia amurensis Rupr*. (Tiliaceae) showed cytotoxic effect against A549, SK-OV-3, SK-MEL-2, and HCT-15 cell lines with IC_{50} values of 3.26 – 8.89 μM and the *Tilia* × *viridis* dichloromethane extract inhibit proliferation of murine lymphocytes cancer cells with an IC_{50} about 3.8 ± 0.2 μg/mL [10, 11]. *P. odoratum* extract has been reported to inhibit the proliferation and induces the apoptosis of human breast cancer MDA-MB-231 cells [13].

Cytotoxic effect of other herbal medicines on AGS and SKOV-3 cancer cell lines has been studied. The leaf extracts of *T. sinensis* was found to have the cytotoxicity effect on SKOV-3 ovarian cancer cells with IC_{50} of 26 μg/ml and inhibition of cell growth was observed in the SKOV3 cell line with ginger extract in the concentrations of 30 μg/ml [22, 23].The IC_{50} of *Saussurea lappa* and *Pharbitis nil* extracts for AGS cells was about 100 and 12.5 mg/ml and extract of *Glycyrrhiza glabra* roots was cytotoxic, and it inhibited AGS cancer cells growth, with IC_{50} value of 40 μM in a dose-dependent manner, also *Rhus verniciflua Stokes* extract had cytotoxic effect on AGS cells with an IC_{50} about 50 μg/ml [24-26].

4. Conclusion

Results of current research indicate that *T. dasystyla* and *P. orientale Desf* extracts have shown cytotoxic effect on SKOV-3 and AGS cancer cell lines due to their polyphenolic content. This preliminary study, in the field of anticancer effect of *T. dasystyla* and *P. orientale Desf* extracts, requires more detailed investigation in subsequent studies. Isolation and purification of effective components with anticancer activities and revelation their benefit in the pharmaceutical and health industries is recommended in future studies.

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