



Research Article

Cytotoxicity Effects of Aqueous Extract of *Purtulaca oleracea* on HeLa Cell Line

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ABSTRACT

Background: Progressive incidence of cervical cancer persuaded the scientists to find the most effective and appropriate treatment approaches such as herbal medicine. Hence, it was aimed in this study to evaluate antitumoral effects of *Purtulaca oleracea* (*P. oleracea*) aqueous extract on HeLa cell line. **Materials:** HeLa cell line was cultured and submitted to different concentrations of *P. oleracea* extract for 24 and 48 hours. Cell proliferation and viability were conducted by MTT assay and trypan blue staining respectively. **Results:** our results showed that cell proliferation was decreased significantly along with increase in *P. oleracea* extract concentrations as well as time lapse ($p < 0.05$). Also, the same result was obtained for cell viability. **Conclusion:** it is suggested that *P. oleracea* aqueous extract has antiproliferative effects on HeLa cell line depending on concentration and time. Though, it appears that this extract decreased the growth of HeLa cells but its cytotoxicity on non-tumoral cells must be considered. Also, it is necessary to know which mechanisms cause its antiproliferative properties.

Introduction

Cervical cancer is occurred when the abnormal cells are undergoing to the rapid and uncontrolled growth on the cervix. It will be highly curable when is being diagnosed at the beginning stage of the disease. Current treatments for cervical cancer may include surgery, drugs (hormonal therapy and chemotherapy), radiation and/or immunotherapy.¹ Conventional cancer treatments such as chemotherapy and radiotherapy have shown some effectiveness for reducing or eradicating cancers; however, they can produce unpleasant side effects, e.g. nausea, vomiting, changes in bowel habits, fatigue and hair loss.² Complementary and alternative medicine (CAM), herbals and multivitamin supplements, or herbal medicine is increasingly used as an adjunctive treatment for cancer patients to reduce or manage side effects of conventional cancer treatments.³ Several studies have confirmed the anti-proliferative and cell cycle regulatory effects of some plants which behave as cancer prevention.^{1,4-6} *Portulaca oleracea* (*P. oleracea*), also known as verdolaga, is an annual succulent in the Portulacaceae family. Several studies have found that *P. oleracea* has a high antioxidant property,^{7,8} vitamins, and dietary minerals, such as magnesium, calcium, potassium, and iron. Furthermore, it has a rare subclasses of

homioisoflavonoids, that their cytotoxic activities have been shown towards human cancer cell lines.⁹

Numerous studies revealed some biological properties of *P. oleracea* such as antiseptic,¹⁰⁻¹² wound-healing effects and⁸ anti-inflammatory activities.¹⁰ In addition, it has been shown that cancer and heart diseases may be reduced by consumption of this plant.¹³ It has been revealed that the aqueous extract of *P. oleracea* has no cytotoxicity or genotoxicity for daily consumption as a vegetable.¹⁴

HeLa is an immortal cell line used in medical research. The cell line was derived from cervical cancer taken from Henrietta Lacks, who died from her cancer in 1951.¹⁵ Antitumoral agents usually exert their effects on cell division process and apoptosis. On the basis of the previous studies regarding to efficacy and *in vitro* cytotoxic activities towards human cancer cell lines of *P. oleracea* extract, the present study was designed to evaluate the cytotoxicity and antiproliferative properties of aqueous extract of the *P. oleracea* on HeLa cell line.

Materials and Methods

Preparation of *P. oleracea* aqueous extract

P. oleracea was purchased from Medical Herb Garden of Hamadan, Iran in summer of 2014. After drying at room temperature for 2 weeks, the dried plant was

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powdered. Then, the ground plant was soxhlet extracted with distilled water at 100° C for 6-8 h and the obtained solution was slowly evaporated to dry.

Cell culture

HeLa cell line was obtained from national cell bank of Iran (NCBI, Pasteur Institute of Iran). The cells were cultured in RPMI-1640 (Sigma-Aldrich,USA) supplemented with 10% fetal bovine serum (FBS) (Gibco/Invitrogen), and 100u/ml penicillin (Sigma-Aldrich,USA) and 100µg/ml streptomycin (Sigma-Aldrich, USA) in 5% CO₂ at 37°C with 95% humidity of air for 2 weeks. The culture medium was changed twice weekly until confluency was achieved. After 80-90% confluency, the cells were trypsinized and cultured in 96-well and 6-well plates to treat with different concentrations of *P. oleracea* extract for MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and trypan blue staining.

MTT Assay

The HeLa cell line was cultured in 96-well plates (10⁴ cells/well). After 24 hours, the cultured cells were treated to concentrations of 0 (as control), 9000 µg/ml, 10000 µg/ml, 11000 µg/ml, 12000 µg/ml, 13000 µg/ml, 14000 µg/ml, 15000 µg/ml and 16000 µg/ml of aqueous extract of *P. oleracea* for 24 and 48 hours. Then, the 100µl of the culture medium was removed and 15 µl MTT (5mg/ml) was added. 3 hours later the wells were substituted with 200 µl of DMSO (dimethyl sulfoxide). The wells were pipetted and read with spectrophotometer at optical density of λ570. Viable cells were measured by:

$$\frac{OD \text{ of experimental groups}}{OD \text{ of control}} \times 100 \quad \text{Eq.(1)}$$

Viability Test

The HeLa cells were cultured in 6-well plates and treated with 13000, 14000, 15000 and 16000 µg/ml of aqueous extract of *P. oleracea* for 24 and 48 hours. Then, the cells stained with trypan blue (Sigma-Aldrich,USA). Briefly, the culture medium was removed and replaced with trypan blue (0.4%). Blue stained cells as dead cells reported.

Statistical analysis

Results were presented as mean ± SD in triplicate experiment. Differences were determined using ANOVA with the Tukey–Kramer multiple comparisons test at significant difference of 0.05.

Results

Effects of *P. oleracea* extract on cell proliferation

Our results revealed that cell proliferation was decreased significantly in all groups submitted with *P. oleracea*. It was dose and time dependent, as, along with increase in concentrations of *P. oleracea* the proliferation of the cells decreased. As it has been presented in Fig 1 the concentration of 16000 µg/ml induced more cell cytotoxicity on HeLa cells after 24 hours. Also, the same results were obtained after 48 hours and the concentration of 16000 µg/ml more inhibited the growth of the cells (P<0.001). With 13000, 14000, 15000 and 16000 µg/ml of *P. oleracea* 50% or more than 50% of the cells died. (Fig 1- Table 1).

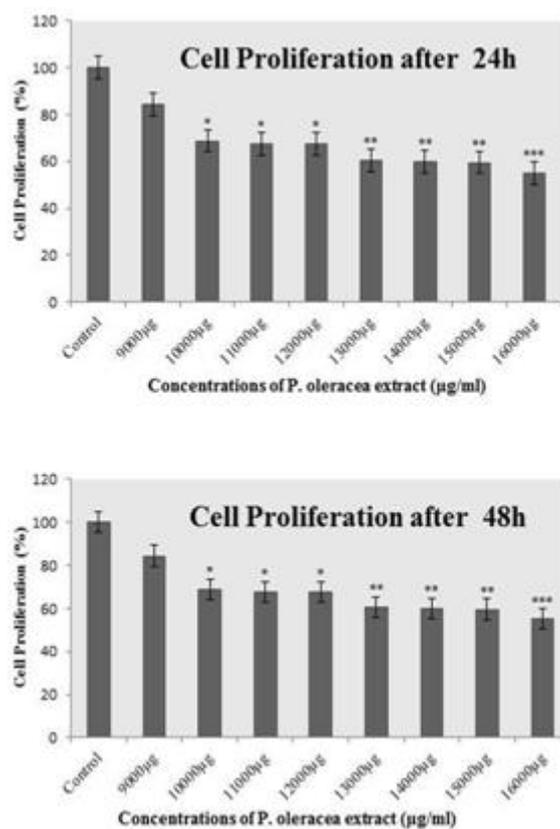


Figure 1. HeLa cell proliferation under treatment with different concentrations of *P.oleracea* in 24h (A) and 48h (B). Cell viability was significantly reduced when the concentrations of *p. oleracea* increased (p < 0.05). Data are mean ± standard deviation. * A significant difference < 0.05, ** A significant difference < 0.01, ***A significant difference < 0.001 toward control group.

Table 1. Cell proliferation after different concentration of *P. oleracea* extract

Cell proliferation (%)	Concentrations of <i>P. oleracea</i> extract (µg/ml)								
	Control	9000	10000	11000	12000	13000	14000	15000	16000
24h	100	84.4±5.1	68.8±8.5	67.5±5.7	67.5±5	60.4±5.7	60±5	59.6±5.7	55±6.4
48h	100	81.8±13.3	60.1±5.8	57.1±11.6	52.75±4.2	45.8±7.5	36.75±10.6	31.75±5	30.8±8.3

Effects of *P. oleracea* extract on cell viability

As it is shown in Fig. 2, 13000, 14000, 15000 and 16000 µg/ml of *P. oleracea* significantly decreased viability of HeLa cells after 24 and 48 hours ($P < 0.001$). Also, cell death was higher after 48h compared to 24h.

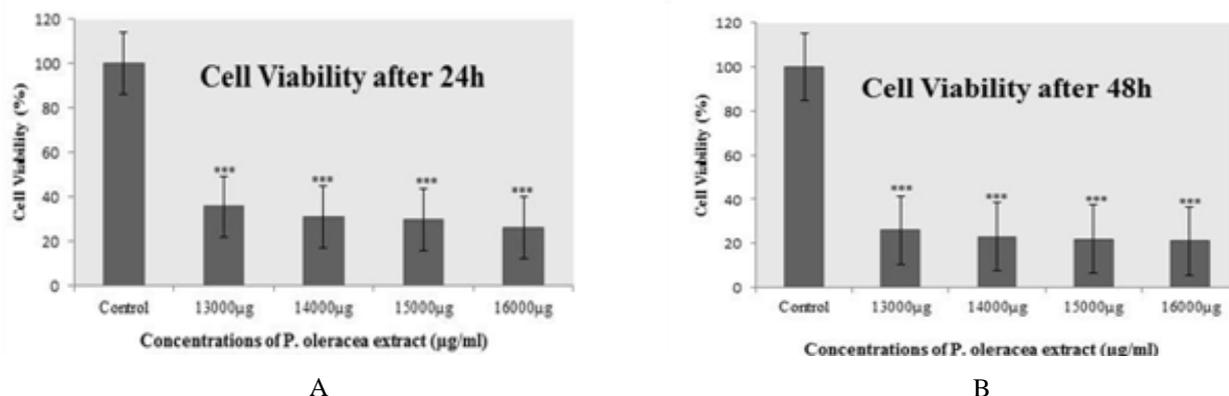


Figure 2. HeLa cell viability under treatment with different concentrations of *P. oleracea* in 24h (A) and 48h (B). Cell viability was significantly reduced when the concentrations of *P. oleracea* increased ($p < 0.001$). Data are mean \pm standard deviation. ***A significant difference < 0.001 toward control group.

Table 2. cell viability after different concentration of *P. oleracea* extract

Cell Viability (%)	Concentrations of <i>P. oleracea</i> extract (µg/ml)				
	Control	13000	14000	15000	16000
24h	100 \pm 0	35.7 \pm 7.5	31.02 \pm 2.3	30.02 \pm 9.2	26.57 \pm 5.8
48h	100 \pm 0	25.5 \pm 3.24	22.6 \pm 4.4	22.27 \pm 2.12	21 \pm 6.5



Figure 3. morphology of HeLa cells. Unstained cells (A), trypan blue staining in control group (B), groups receiving the extract with more dead cells presented as blue cells (C and D).

Discussion

Recently, many researchers are focusing on cancer prevention, detection and treatment. Finding of the approaches to reduce the risk of cancer is essential and is the world tendency in this field.

The present study examined the cytotoxicity effects of *P. oleracea* aqueous extract on HeLa cell line. Overall, our findings confirmed the antitumoral effects of *P. oleracea* based on MTT assay and also trypan blue staining. It was noted that it exerted its property through dose and time dependant manner. While, the

As it was mentioned in MTT results, concentration of 16000 µg/ml of *P. oleracea* had more toxicity on cells compared to other doses at 24 and 48 h. (Fig. 2 and 3) (Table 2).

apoptosis was not performed in the experiment but it is suggested that cell death might be occurred through apoptosis.

P. oleracea is reported as a rich source of antioxidant and omega-3 fatty acids.^{6,16}

Omega-3 fatty acid may have an important role in the protection against cardiovascular disease and cancers. As well as, it is reported that the antioxidants present in *P. oleracea* can act against oxidative stress¹⁷. It is suggested that removing of free radicals produced in cancer might be the one of the mechanisms that *P. oleracea* exerts its antitumoral effects.

The potential anti-proliferative activities of *P. oleracea* methanol extract towards various types of cancerous cells has been determinate using of MTT assay, BrdU incorporation assay and flow cytometry RNase/PI staining to quantify on the cell cycle progression.⁶

Zakaria et.al found that aqueous extract of *P. oleracea* has antiproliferative efficacy against AMN3 cell line in a dose and time dependent manner, too¹⁸. Moreover, it has been confirmed that after increasing the concentration of *P. oleracea* to 100 µg/ml, more HEPG2 cells faced to cell death and these results showed the cytotoxic effect of *P. oleracea* on HEPG2 cell line¹⁹. Furthermore, it is reported that this plant have antihyperglycemia²⁰, antibacterial²¹, hepatoprotective²² and even bronchodilatory effects²³. Therewith, Chen et al. suggested that water soluble polysaccharides of *P. oleracea* possessed mild

cytotoxic activity against cervical cancer HeLa cell line and the sulphated form of these polysaccharides enhanced the anti-tumour effect^{6,24}.

Antitumoral property of *P. oleracea* was assigned to antiproliferative, apoptotic and cytotoxic agents such as flavonoids, alkaloids and anthraquinones coumarins found in it¹⁷. It is confirmed that dietary flavonoids cause to inhibit the proliferation of various cancer cells and tumor growth in animal models, as well as protect against cancer induction in several human tissues¹⁸. Also, it is reported that several flavonoids regulate the critical genes for the control of proliferation, cell cycle and apoptosis pathway in cancer cells.

Conclusion

According to obtained results of present study could be concluded that *P. Oleracea* aqueous extract exhibit antiproliferative and apoptotic effects against HeLa cell line in a dose and time dependent manner. We suggest that *P. oleracea* aqueous extract can act as an anticancerous herbal drug.

Conflict of Interest

The authors report no conflicts of interest.

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