

ARTICLE

CYTOTOXICITY EFFECTS OF ETHANOLIC EXTRACT OF AERIAL PARTS OF NASTURTIUM OFFICINALIS ON HELA CELL LINE

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ABSTRACT

Background: Today, there has been growing interest in developing natural products for anticancer drugs due to their diverse pharmacological properties and benefits. In this study, we decided to assess the cytotoxic effect of the ethanolic extract of aerial parts of *N. officinalis* on HeLa cells. **Methods:** HeLa cancer cell line was obtained from National Cell Bank of Iran and cultured in RPMI. The ethanolic extract of aerial parts of *N. officinalis* was applied in 8 different concentrations. To evaluate the cytotoxicity effect of the extract on HeLa cell line, MTT colorimetric assay was applied. **Results:** The highest percentage of cell growth inhibition was 91.8% at 1.25 mg/ml concentration. The IC₅₀ was measured as 0.305 mg/ml. **Conclusion:** Based on the results of this study, the ethanolic extract of aerial parts of *N. officinalis* has cytotoxic effects on the HeLa cell line. Isolation of effective compounds of this extract and evaluation of their effects on tumor-bearing animal models are necessary.

INTRODUCTION

KEY WORDS
cytotoxic, *Nasturtium officinalis*, HeLa

The conventional modality for cancer therapy by radiation and chemotherapy cause serious side effects like fatigue, diarrhoea, nausea, hair loss, skin problems, malfunction of urinary bladder and decrease in RBCs due to cytotoxicity and genotoxicity of radiation and chemotherapeutic agents on the non-tumor cells [1]. A successful anticancer drug is one that kill or in capacitate cancer cells without causing excessive damage to normal cell [2]. The plant-based anticancer drugs are being remarked by scientists due to their diverse pharmacological properties and benefits. Currently 25% of all prescription drugs are derived from natural sources and for anti-cancer drugs, more than 80% are plant-derived compounds [3]. The plant-derived anticancer drugs act via different pathways, which ultimately result in activation of apoptosis of cancer cells leading to cytotoxicity [4].

Cruciferous Vegetables are a member of the family of Brassicaceae or cabbage family that include Kale, *Nasturtium officinalis* (Watercress), broccoli, cabbage, Brussels sprouts and turnips. *Nasturtium officinale* are fast growing, aquatic or semi-aquatic, perennial plants native from Europe to central Asia, and one of the oldest known leaf vegetables consumed by human beings [5]. This herb is used to treat diabetes, bronchitis, and diuresis, as anti-ulcerogenic, in treatment of scurvy, tuberculosis, influenza, asthma, nutritional supplement and digestive aid and also seems to have antimicrobial, anticarcinogenic, and antiestrogenic activity [6].

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N. officinalis contains oils, like mustard oil [7] and isothiocyanates such as phenylisothiocyanate that have cancer-preventive [8]. Glucosinolates like gluconasturtiin and carotenoids like lutein are also found in *N. officinalis* [9]. A high dietary intake of *N. officinalis* has been linked to a reduction in cancer [10] and reduces the DNA damage in white blood cells and increases the antioxidant uptake [11]. The crude extract of *N. officinalis* has demonstrated significant anti-genotoxic, anti-proliferative and anti-metastatic potential in human colon cancer cells^[12], human oral cancer [5] and human MDA-MB-231 breast cancer cells [10]. Considering the phytochemical and pharmaceutical bioactive component of *N. officinalis* in medicinal use, little evidence exists showing the possible effects of *N. officinalis* on cancer cell line. However in this study, we evaluated the cytotoxic effect of the ethanolic extract of *N. officinalis* on HeLa cell line.

MATERIALS AND METHODS

Cell culture

HeLa (NCBI C115) cancer cell line was obtained from National Cell Bank of Iran (the Pasteur Institute of Iran, Tehran) and cultured in RPMI 1640 (purchased from PAA) supplemented with 10% FBS, 100 U/ml penicillin and 100 µg/ml streptomycin. They were incubated in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C.

Collection of Plant material and preparation of ethanolic extract

The plant material was identified by Dr. Bahman Eslami (Assistant. Prof. of plant system, Islamic Azad University of Qaemshahr, Iran). Voucher specimens are deposited with the faculty of biology herbarium (as No 720 - 202). The leaves, stems and flowers were collected in the month of February and March 2013 from around Amol region (Latitude 36.26 N longitude 52.24 E). Aerial parts of the plant was thoroughly washed with water followed by shade dried at room temperature and powdered by using mortar and pestle. The

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powdered plant material was percolated with ethanol (96%) for 3 days. The ethanolic extracts were then filtered and concentrated by indirect heat.

MTT assay

To evaluate the cytotoxicity effect of the ethanolic extract of *N.officinalis* on the Hela cancer cells, MTT colorimetric assay was used. In short, cells (10000 cells/ml) were transferred into 96-well culture plates and incubated for 24 h. Then 100 ml of various concentrations of extract of *N.officinalis* (10,7.5,5,2.5,1.25,0.625,0.312,0.156 mg/ml) were added and the micro plates were further incubated for 72 h. Dilution of stock solutions was made in culture medium with a final DMSO conration of 0.1% . Untreated cancer cells was used as a positive control. For testing the effect of extract on normal cells , we use peripheral blood mononuclear cells at 1×10^4 /ml in RPMI 1640 .After 72 hours, MTT assay was performed. In this assay mitochondrial enzyme of viable cells reduces metabolically the soluble MTT into an insoluble colored formazan product which in turn can be dissolved in DMSO and measured spectrophotometrically [13]. To evaluate cell survival, each well was incubated with 20 ml of MTT solution (5 mg/ml in PBS) for 3 h and after removing of well content, DMSO was added to well and mixed to dissolve insoluble formazan crystals. Cells were incubated further to allow for color development, then the absorbance values were read at 492 nm using an ELISA plate (Awareness,USA) and was calculated using the equation: (mean OD of treated cells - OD blank/mean OD of control cells - OD blank) \times 100.Also the rates of IC₅₀ were measured.

Observation of morphological changes

Morphological changes in HeLa cells treated with the 1.25 mg/ml concentration of ethanolic extract were monitored under an inverted microscope (Motic AE 31 .Australia) and compared with the control group after 72 h treatment.

Statistical Analysis

Data was expressed as mean \pm S.E.M. Statistical analysis was performed with Student's t-test using the independent t-test. Differences were considered significant at $P \leq 0.05$.The (Inhibition Concentration) IC₅₀ value was obtained from MTT assay and calculated using Microsoft excel software from linear regression analysis.

RESULTS

Cytotoxicity assay

To evaluate the cytotoxicity effect of the ethanolic caxtract of *N.officinalis*, viability tests were applied using MTT assay. [Table 1] shows the percentage of growth inhibition of the treated cells with different concentration of ethanolic extract of *N.officinalis* on Hela cells line. The highest percentage of cell growth inhibition at 1.25 mg/ml concentration was 91.8% [Fig. 1 and Table 1] and the rate of IC₅₀ was measured as 0.305 mg/ml. The significant decrease in cell growth ($p \leq 0.05$) was observed for 0.625 ,1.25 ,2.5 ,5 ,7.5 ,10 mg/ml ,when compared to untreated control cells [Table 1].Treating of normal cells with 10 mg/ml concentration of extract show significantly decrease in cell growth when compared with control group.

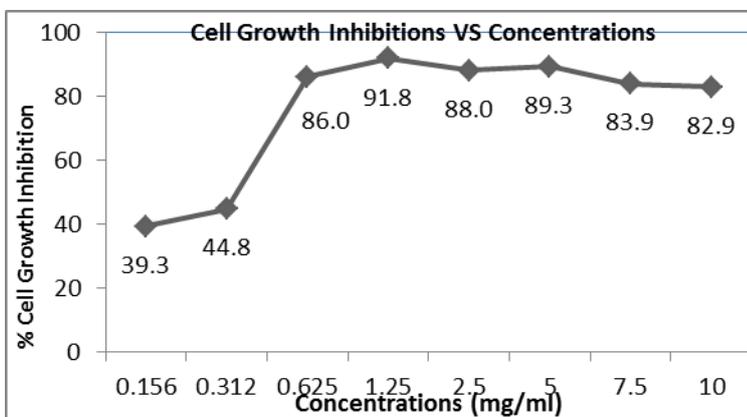


Fig. 1: Growth inhibition percentage of Hela cells in different concentration of ethanolic extract of *N.officinalis*

Table 1: Cytotoxicity results of ethanolic extract of *N.officinalis* on Hela cell at different concentrations by MTT assay

Concentratipons of <i>N.officinalis</i> (mg/ml)	Absorbance (mean± sem)	Inhibition %	IC ₅₀ (mg/ml)
Control	0.257 ± 0.049		
0.156	0.172 ± 0.060	39.353	
0.312	0.151 ± 0.029	44.822	0.305
0.625	0.060 ± 0.013 *	86.079	
1.25	0.047 ± 0.014 *	91.080	
2.5	0.051 ± 0.003	88.038	
5	0.049 ± 0.002	89.311	
7.5	0.060 ± 0.008 *	83.931	
10	0.074 ± 0.016 *	82.999	
DMSO	0.224 ± 0.070	1.3	
Lymph	0.080 ± 0.005	75.27	

Morphological studies

The morphological changes were obtained comparison to control cells under the invert microscop (Magnificantion 200x)[Fig.2]. After 72h,the morphological characteristics of apoptotic cell death, such as cell shrinkage, condensation,vacuolation and pigmentation was observed.

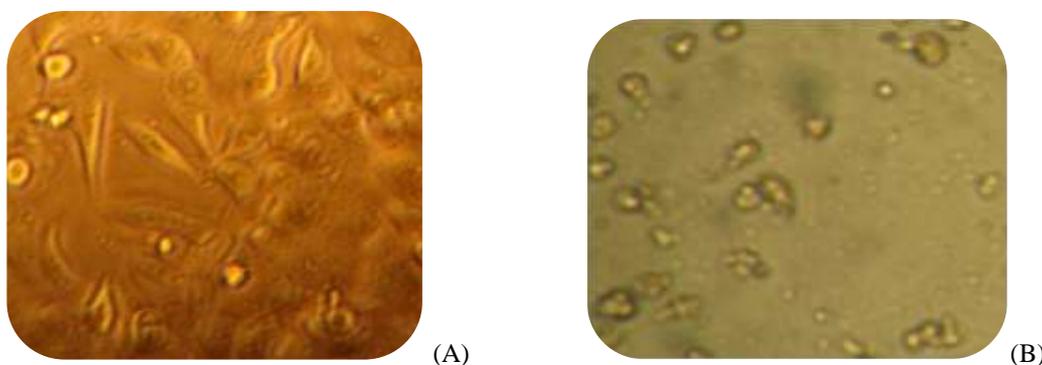


Fig.2: (A) Control Hela cells.(B) Hela cells treated with ethanolic extract of *N.officinalis* after 72h.Results revealed that morphological changes were typical of apoptosis

DISCUSSION

Cervical cancer is a malignant neoplasm of the cervical area. It is an important women’s health problem in developing countries. Herbal medicines in all regions of the world are widely used for treatment of disease especially cancer. Today, more than 85000 plant species have been documented for medical use [14] . There have been several researches to get new cytotoxic agents. In this regard compounds such as Camptothecin, Taxol, Combretastatin, Podophyllotoxin, colchicine, Vinca alkaloids and paclitaxel isolated from medicinal plants showed considerable promises [15].To evaluate the cytotoxicity of one of native plants of Iran, this study was conducted. This in-vitro study was undertaken to demonstrate the effects of ethanolic extract of *N.officinalis* on Hela cells by MTT assay. we showed that treatment of the Hela cells with 0.625 , 1.25 , 2.5 , 5 , 7.5 , 10 mg/ml concentrations of ethanolic extract resulted in a significant ($p \leq 0.05$) increase in the cell death and the morphological characteristics of apoptotic cell death . Normal cells at 10 mg/ml concentrations of extracts were damaged.

Several studies on cytotoxic effects of *N.officinalis* in vitro and in vivo , are shown toxicity of this extract on cancer cells .A report by Rajalakshmi and Agalyaa showed antitumor activity of extract of *N.officinalis* on oral cancer. They showed that Phenethyl Isothiocyanate (PEITC) present in *N. officinale* acts as a chemo preventive agent and inhibit bind of PEITC to Cytochrome P450 [18].

Rose and et al have shown that a high dietary intake of cruciferous vegetables has been associated with a reduction in numerous human pathologies particularly cancer. They show that the ability of cruciferous vegetables to chemo prevention of cancer cells attributed to inhibit cancer cell proliferation, invasion , and metastatic potential [16].

Another group of active constituents of these plants are glucosinolates. The glucosinolates are a large group of sulphur-containing glucosides found in cruciferous vegetables. Glucosinolates are broken down to glucose and isothiocyanates (ITC). ITC, the most important products of broken glucosinolates, can prevent cancer by blocking DNA damage, inhibiting the growth of tumor cells, stimulating apoptosis, inhibiting mitosis, inducing cell cycle arrest, and promoting of apoptosis [17].

Previous studies have shown that ITCs can inhibit the NF-KB signaling pathway in several cancer cell and in animal model [18-21]. Moreover, another evidence from Zhu and et al has also demonstrated that the ITC sulforaphane can inhibit AP-1 DNA binding in human keratinocyte exposed to UV-B irradiation [22].

Carotenoids like β -carotene and lutein also present in these vegetables. Lutein in addition of protecting the retina from photooxidative damage, can prevent cancer. Lutein has also shown antimutagenic and anticarcinogenic invivo and invitro by regulating apoptosis [17].

In the present study, we showed cytotoxicity effect of ethanolic extract of *N.officinalis* on Hela cells. Also morphological characteristics of apoptotic cell death, such as shrinkage, condensation, vacuolation and pigmentation is observed. These results may be due to the presence of some bioactive compounds in this extract such as Glucosinolates , Phenethyl Isothiocyanate or carotenoids that leads to increased apoptosis in these cells. Therefore *N. officinalis* can be a natural potent chemopreventive and chemotherapeutic plant. Further studies are necessary to assess bioactive compounds of *N.officinalis* to improve the efficacy and testing on the other cell lines (human).Also further molecular studies need to elucidate the mechanisms of action of these extract on cancer cells.

CONFLICT OF INTEREST

There is no conflict of interest

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None

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