Cytotoxic Activity of *Rosa Damascene Mill, Allium sativum, Allium Hirtifolium Boiss, and Prosopis Farcta* Extracts on Human Cervical Carcinoma Cell Line

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Abstract

**Background:** Cervical cancer is one of the major reasons of cancer-related mortality. Human papillomavirus is the most common sexually transmitted viral infection, which can lead to cervical cancer. There is no powerful chemotherapeutic agent for HPV infection and cervical cancer. Some plants have the proper potential to be used for treatment of cervical cancer caused by HPV type 18.

**Materials and Methods:** In this study, cytotoxic effect of extract of four indigenous Iranian plants including *Rosa damascene mill, Allium sativum, Allium hirtifolium boiss* and *Prosopis farcta* were investigated on the HeLa cell line. HeLa cells were incubated with different concentrations of extracts and then the cell viability was measured by MTT assay.

**Results:** The viable cell numbers were decreased by increase of the extracts concentration. The *Allium sativum* showed the higher cytotoxicity in all concentrations than the other ones. Afterwards, *Allium hirtifolium Boiss, Rosa damascene mill, and Prosopis farcta* showed maximum efficiency to decrease cell viability, respectively.

**Conclusion:** The above four mentioned plants might be used for death of HeLa cell harboring HPV type 18. Therefore, they could be employed as a chemotherapeutic agent in the cervical cancer treatment in future.

**Keywords:** *Rosa damascene mill, Allium sativum, Allium hirtifolium boiss, Prosopis farcta*, Cervical cancer, Cytotoxicity

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**Introduction**

Recent advancement in molecular biology of viruses has improved the knowledge about cell transformation and oncogenesis. Viruses play a significant role in changing the dynamics of non-communicable diseases including cancers. They are implicated in about 20% of human cancers and are identified as second risk factor in human cancerology¹. Human papillomavirus (HPV), a double-stranded...
DNA virus from the *Papillomaviridae* family is considered as the most common sexually transmitted viral infection. Infection is commonly found in sexually active women, even among those thought to be at lower risk of developing other sexually transmitted infections. HPV are implicated in more than 5.2% of the infectious-related cancers and almost all cases of cervical cancer. Also, it is the second most frequently reported cancer which causes 42% mortality especially in developing countries. Only certain HPV types (especially 16 and 18) have been strongly associated with anogenital cancers particularly cervical cancer. Studies have demonstrated that persistent cervical infections with high-risk HPV preceed the appearance of the precursor lesions and are required for the development, maintenance and progression of these lesions.

Even though cervical cancer is almost 100% preventable using Pap screening and bivalent, tetravalent or nine-valent HPV vaccines, it remains as a public health challenge since the vaccines do not have accessible for majority of the subjects. In addition, there is no potent chemotherapeutic agent for HPV infection, the precursor lesions as well as cervical cancer. Furthermore, it requires multiple therapeutic approaches including pharmacological and non-pharmacological (surgery and radiotherapy) treatments to manage the cervical cancer, depending on the clinical staging and general condition of the patient. These strategies are accompanied with the adverse effects including bone marrow suppression that further worsens the condition. Consequently, it has become an important issue for both researchers and clinicians to develop a novel anticancer agent(s) to treat the HPV infection. However, with advancement in ethno-medicine, more potent anticancer agents are being discovered, which appear to be promising when compared with the traditionally used cytotoxic agents.

A large number of plants products have been identified for tumor treatment through various mechanisms including immune activation, induction of apoptosis, DNA repair and altered metabolism in transform cells. Traditionally, antiviral agents such as *Asarum* heterotropoides are used for HPV infection.

In this study, we aimed to identify and evaluate cytotoxic activity of some indigenous herbal including *Rosa damascene mill, Allium sativum, Allium hirtifolium Boiss,* and *Prosopis farcta* on the human cervical carcinoma cell lines harboring HPV type 18. They are used traditionally in organized traditional medical systems in Iran for treatment warts, infections, inflammatory conditions and cancers.

**Methods**

**Extracts preparation:** The indigenous Iranian plants including *Rosa damascene mill, Allium sativum, Allium hirtifolium boiss* and *Prosopis farcta* materials were collected from different regions. Then, they were finely grounded after drying at room temperature in a dark condition. 100 g of the powdered materials (*Rosa damascene mill, Allium hirtifolium Boiss,* fruit of *Prosopis farcta* and Allium *sativum*) were suspended in boiling water, individually. After 30 minutes, they were filtered and the solvent was evaporated with vacuum rotary evaporator (Heidolph Germany). Subsequently, the extracts were sterilized via filtration with 0.22μm pore sized sterile filters. Final concentrations ranging from 3 to 9 μg/ml were prepared with dimethyl sulfoxide (DMSO).

**Cell preparation:** Human cervical cancer cell line, Hela cells, harboring HPV type 18 was used in this study. Minimum essential medium (MEM) supplemented with 10% heat inactivated fetal calf serum and 1% penicillin-streptomycin mixture was used to grow and harvest the cell in the monolayers. Maintenance conditions were 37°C, 100% relative humidity, and 5% CO2.

**Cytotoxicity assay and cells viability:** The cytotoxic activity of the plants’ extracts was checked using a modified 3-(4,5-dimethyl thiazole-2-yl)-2,5-diphenyl tetrazolium (MTT) assay as previously described. Cells viability was determined using tryptophan blue via absorption capacity and the cells were categorized.

**Statistical Analysis:** Statistical analysis was conducted using Prism version 7.0. An ordinary one-way (ANOVA) was used to compare each concentration to 0 μg/ml of four plant extracts.
Results

We selected four indigenous herbals including *Allium sativum*, *Rosa damascene mill*, *Allium hirtifolium Boiss*, and *Prosopis farcta*. The possible cytotoxic activity of plant extracts on HeLa cells, harboring HPV type 18, the cytotoxicity on HeLa cells measured by microculture tetrazolium (MTT) assay. Figure 1 shows the cell number before and after treatment by three concentrations (3, 6, and 9 µg/ml) of plant extracts. The viable cell numbers decreased by increase of the extracts concentration. The statistical analysis revealed that the significant differences were found between untreated samples and treated samples with 9 µg/ml of plant extracts (p=0.014, 0.007, 0.007 and 0.009 for *Rosa damascene mill*, *Allium sativum*, *Allium hirtifolium Boiss*, and *Prosopis farcta*, respectively). Moreover, figure 2 demonstrates the percent of cell viability in the presence of the plant extracts in various concentrations. The *Allium sativum* showed the higher cytotoxicity in all concentrations than the other ones. The cell viability percentage after treatment by other plant extracts decreased as follows: *Allium hirtifolium Boiss*, *Rosa damascene mill*, and *Prosopis farcta*.

Discussion

The results obtained in this study revealed that the extracts of *Allium sativum*, *Rosa damascene mill*, *Allium hirtifolium Boiss*, and *Prosopis farcta* produce dose-dependent inhibitory activities against HeLa cells. Among the four plant extracts, *Allium sativum* extract showed highest growth inhibitory effect in HeLa cells. The cytotoxic screening model provides preliminary data for selecting potent herbal remedies by their potential benefits as anticancer agents and to be used both in human and nonhuman cancer treatments.

More than 32 million people carry different HPV types in Asia, of which 3.7 million (12%) live in Iran. Moreover, the cervical cancer has sixth rank in terms of prevalence among all cancers according to Iranian cancer registry. Nowadays, the burden of HPV associated cancers is accentuated in the young age, therefore the psychological and financial distress in controlling the diseases are important. The major problems encountered in the cervical cancer chemotherapy arose from the side effects of the agent in use because of non-selectivity and resistance.
Therefore, search and development of new and efficient anticancer agents have become very fundamental issue. Allium genus belongs to monocots flowering plants comprising hundreds of species. Since now, the presence of several compounds including saponins, sapogenins, flavonoids, thiosulfimates, disulfide, and trisulfide were reported in the Allium genus species.

Allium sativum (garlic) is an ancient plant that was used for treating different diseases such as arthritis, heart disease, abdominal growths, diarrhea and worm infestation\(^\text{14}\). The daily consumption of garlic can diminish the cancer risk. Moreover, its extract can effectively blocked the induced tumors in breast, uterine cervix, skin, and colon\(^\text{14, 15}\).

Allium hirtifolium Boiss (Persian Shallot) is an Iranian native plant that is usually employed for treatment of rheumatoid, hypertension, inflammation, and healing of wounds\(^\text{16}\). Moreover, the properties of antifungal, antibacterial, and anticancer of Allium hirtifolium Boiss have been reported\(^\text{16, 17}\). Prosopis farcta is an Asian native plant, which is used by local native people in the treatment of diarrhea, colds, inflammation, skin diseases, and prostate disorders\(^\text{18}\). Rosa damascena mill belongs to genus Rosa and Rosaceae family, which is rich of flavonoid and leads to improve the cardiovascular function\(^\text{19}\). In addition, it has anti-diabetic activity, protective effects on neuritic atrophy, depression and stress improvement, and hypnotic effect. Moreover, cytotoxic effect of Rosa damascena on cancer cells of human lung, breast cancer and cervix cancer have been reported\(^\text{20, 21}\).

### Conclusion

In this study, we showed the cytotoxicity effect of Allium sativum, Allium hirtifolium Boiss, Rosa damascena mill, and Prosopis farcta on the HeLa cell harboring HPV type 18. Our findings serve as basis for future research to establish how these plants’ product produce the desired effect. We suggest that these plants could be a source for new lead structures in treatment.
design anti cervical cancer drugs.

Acknowledgment

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References