

Original Article

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 infection². Infection is commonly found in sexually active women, even among those thought to be at lower risk of developing other sexually transmitted infections³. HPVs are implicated in more than 5.2% of the infectious-related cancers and almost all cases of cervical cancer⁴. Also, it is the second most frequently reported cancer which causes 42% mortality especially in developing countries⁵. 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 HPVs precede the appearance of the precursor lesions and are required for the development, maintenance and progression of these lesions^{6, 7}.

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^{8, 9}. 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^{9, 10}. Traditionally, antiviral agents such as *Asarum heterotropoides* are used for HPV infection¹⁰.

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 categorized¹¹.

Statistical Analysis: Statistical analysis was determined 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 *Prosopisfarcta*. 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 *Prosopisfarcta*, 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 *Prosopisfarcta* 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^{12, 13}. Nowadays, the burden of HPV associated cancers is accentuated in the young age, therefore the psychological and financial distress in controlling the diseases are important⁵. 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.

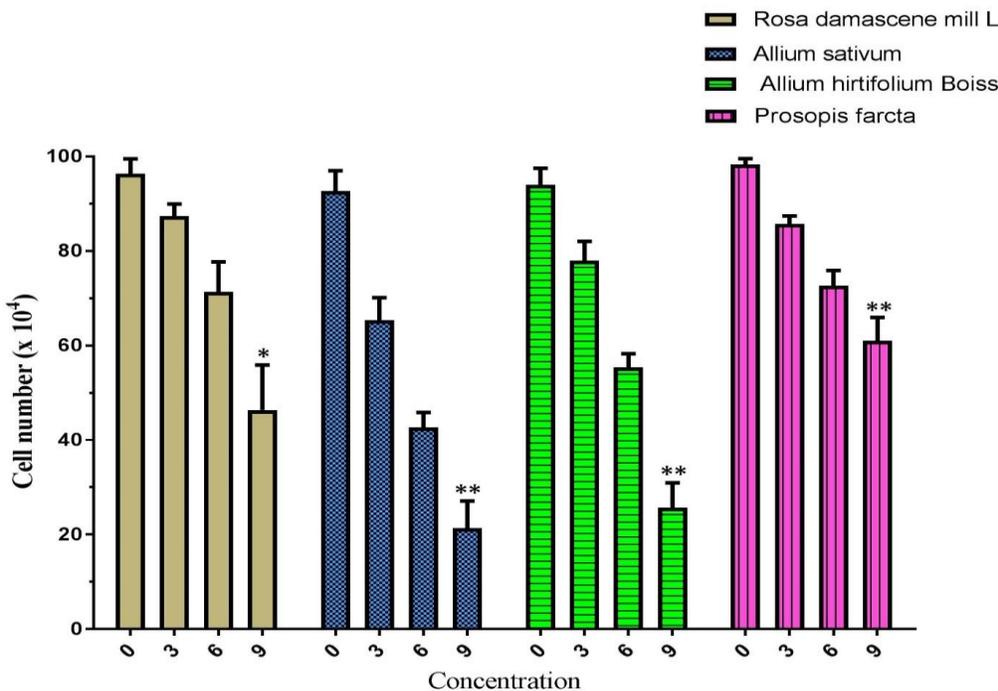


Figure 1: The cell number before and after treatment by three concentrations (3, 6, and 9 µg/ml) of plant extracts

Therefore, search and development of new and efficient anticancer agents have become very fundamental issue.

Allium genus belongs to monocotyledonous flowering plants comprising hundreds of species. Since now, the presence of several compounds including saponins,

sapogenins, flavonoids, thiosulfinates, disulphide, and trisulphide 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 infestation¹⁴. 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^{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 wounds¹⁶. Moreover, the properties of antifungal, antibacterial, and anticancer of *Allium hirtifolium* Boiss have been reported^{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 disorders¹⁸.

Rosa damascena mill belongs to genus *Rosa* and *Rosaceae* family, which is rich of flavonoid and leads to improve the cardiovascular function¹⁹. In addition, it has anti-diabetic activity, protective effects on neuritic atrophy, depression and stress improvement, and hypnotic effect. Moreover, cytotoxic effect of *Rosa damascene* on cancer cells of human lung, breast cancer and cervix cancer have been reported^{20,21}.

Conclusion

In this study, we showed the cytotoxicity effect of *Allium sativum*, *Allium hirtifolium* Boiss, *Rosa damascene 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

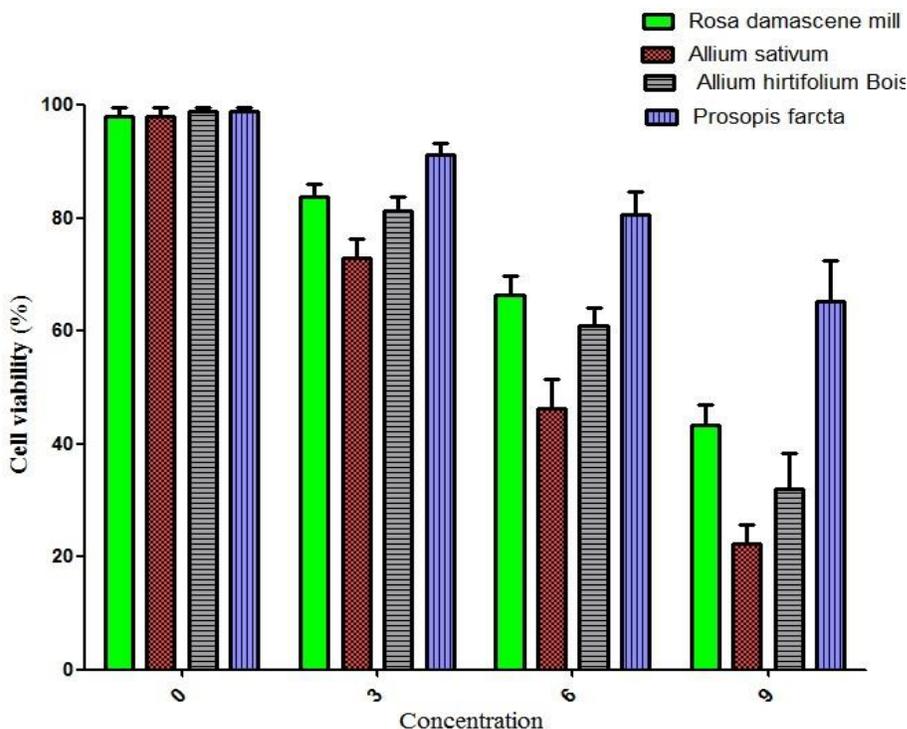


Figure 2: The percent of cell viability in the presence of the plant extracts in various concentrations

design anti cervical cancer drugs.

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References

1. Young LS, Dawson CW. Epstein-Barr virus and nasopharyngeal carcinoma. *Chinese journal of cancer*. 2014;33(12):581.
2. Sawyer BG, Esquivel D, Smith C, Carter TY. The Detection of HPV-Infected Cells Using Real-time PCR. *Clinical Laboratory Science*. 2015;28(3).
3. Moscicki A-B. Impact of HPV infection in adolescent populations. *Journal of Adolescent Health*. 2005;37(6):3-9.
4. Maggino T, Casadei D, Panontin E, Fadda E, Zampieri M, Dona M, et al. Impact of an HPV diagnosis on the quality of life in young women. *Gynecologic oncology*. 2007;107(1):175-9.
5. Insinga RP, Glass AG, Rush BB. The health care costs of cervical human papillomavirus-related disease. *American journal of obstetrics and gynecology*. 2004;191(1):114-20.
6. Forman D, de Martel C, Lacey CJ, Soerjomataram I, Lortet-Tieulent J, Bruni L, et al. Global burden of human papillomavirus and related diseases. *Vaccine*. 2012;30:12-23.
7. Vinodhini K, Shanmughapriya S, Das BC, Natarajaseenivasan K. Prevalence and risk factors of HPV infection among women from various provinces of the world. *Archives of gynecology and obstetrics*. 2012;285(3):771-7.
8. Chao A, Lin C-T, Lai C-H. Updates in systemic treatment for metastatic cervical cancer. *Current treatment options in oncology*. 2014;15(1):1-13.
9. Eskander RN, Tewari KS. Chemotherapy in the treatment of metastatic, persistent, and recurrent cervical cancer. *Current Opinion in Obstetrics and Gynecology*. 2014;26(4):314-21.
10. Taraphdar AK, Roy M, Bhattacharya R. Natural products as inducers of apoptosis: Implication for cancer therapy and prevention. *Current science*. 2001:1387-96.
11. Tiwary BK, Bihani S, Kumar A, Chakraborty R, Ghosh R. The in vitro cytotoxic activity of ethno-pharmacological important plants of Darjeeling district of West Bengal against different human cancer cell lines. *BMC complementary and alternative medicine*. 2015;15(1):22.
12. Jahdi F, Khademi K, Khoei EM, Haghani H, Yazdanpanahi Z. Evaluation of the individual and medical factors associated with genital human papillomavirus in Iranian women. *Scimetr*. 2014;2(4).
13. Kawana K, Yasugi T, Taketani Y. Human papillomavirus vaccines: current issues & future. *Indian Journal of Medical Research*. 2009;130(3):341.
14. Oommen S, Anto RJ, Srinivas G, Karunakaran D. Allicin (from garlic) induces caspase-mediated apoptosis in cancer cells. *European Journal of Pharmacology*. 2004;485(1-3):97-103.
15. Islam MS, Kusumoto Y, Al-Mamun MA. Cytotoxicity and Cancer (HeLa) Cell Killing Efficacy of Aqueous Garlic (<i>Allium sativum</i>) Extract. *Journal of Scientific Research*. 2011; 3(2).
16. Hosseini FS, Falahati-Pour SK, Hajizadeh MR, Khoshdel A, Mirzaei MR, Ahmadirad H, et al. Persian shallot, *Allium hirtifolium* Boiss, induced apoptosis in human hepatocellular carcinoma cells. *Cytotechnology*. 2017;69(4):551-63.
17. Ghodrati Azadi H, Ghaffari SM, Riazi GH, Ahmadian S, Vahedi F. Antiproliferative activity of chloroformic extract of Persian Shallot, *Allium hirtifolium*, on tumor cell lines. *Cytotechnology*. 2008;56(3):179-85.
18. Omidi A, Ansari nik H, Ghazaghi M. *Prosopis farcta* beans increase HDL cholesterol and decrease LDL cholesterol in ostriches (*Struthio camelus*). *Tropical animal health and production*. 2013;45(2):431-4.
19. Zamiri-Akhlaghi A, Rakhshandeh H, Tayarani-Najaran Z, Mousavi SH. Study of cytotoxic properties of *Rosa damascena* extract in human cervix carcinoma cell line. *Avicenna Journal of Phytomedicine*. 2011;1(2):74-7.
20. Haghi G, Hatami A. Simultaneous quantification of flavonoids and phenolic acids in plant materials by a newly developed isocratic high-performance liquid chromatography approach. *Journal of agricultural and food chemistry*. 2010;58(20):10812-6.
21. Zu Y, Yu H, Liang L, Fu Y, Efferth T, Liu X, et al. Activities of ten essential oils towards *Propionibacterium acnes* and PC-3, A-549 and MCF-7 cancer cells. *Molecules*. 2010;15(5):3200-10.