Study of cytotoxic properties of *Rosa damascena* extract in human cervix carcinoma cell line

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Abstract

Objective: Cancer is a major health problem worldwide and current therapies for cancer are often limited by short-term efficacy due to drug resistance. There has been much interest in the use of naturally occurring compounds with chemo-preventive and chemotherapeutic properties in the treatment of cancers.

Rose is one of the most important groups of ornamental plants which their fruits and flowers are used in a wide variety of foods, nutritional products and different traditional medicines.

Material and methods: In this study cytotoxic effect of Rosa damascena extract was evaluated on HeLa cell line. HeLa cells were cultured in DMEM medium and incubated with different concentrations of *Rosa damascene* (*R. damascene*) extract. Cell viability was quantitated by MTT assay.

Results: *Rosa* decreased cell viability in malignant cells in a concentration and time dependent manner. The IC50 values against HeLa were determined as 2135, 1540 and 305.1 $\mu g.ml^{-1}$ after 24, 48 and 72h, respectively.

Conclusion: It might be concluded that *R. damascena* could cause cell death in HeLa cells which could be also considered as a promising chemotherapeutic agent in cancer treatment in future.

Keyword: *Rosa damascena*, Cytotoxicity, HeLa

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Introduction

Rosa damascena (*R. damascene*) is a plant that belongs to genus *Rosa* and Family *Rosaceae*. The plant is shown to have a high level of flavonoids (Haghi and Hatami, 2010). *R.damascena* has significant antioxidant activity and shows potential oxidative prevention from DNA damage (Kalim et al., 2010). It has antibacterial activity towards P. acnes (Zu et al., 2010). *Salmonella typhimurium, Bacillus cereus*, *Candida albicans* and *Methicillin*-resistant *Staphylococcus aureus* (Talib and Mahasneh, 2010). The flavonoids of *R. damascena* improve the cardiovascular function (Kwon et al., 2010). It may have anti-diabetic activity and can reduce the postprandial glucose level (Gholamhoseinian et al., 2009). *R. damascena* has protective effects on neuritic atrophy (Awale et al., 2009). It's essential oil can retard the development of seizure stages (Ramezani et al., 2008) and reduces autonomic arousal (decrease in breathing rate, blood oxygen saturation and systolic blood pressure) which can improve depression and stress in humans (Hongratanaworakit, 2009). The hypnotic effect is also shown in mice (Rakhshandah and Hosseini, 2006).

As the plant has a significant antioxidant activity and potentially preventive actions from oxidative DNA damage (Kalim et al., 2010), it may have antitumor activity. Cytotoxic effect of *R.damascena* on cancer cell lines has been reported on human lung (A549) and breast cancer (MCF-7) cell lines (Zu et al., 2010). In this study, the cytotoxic effect of *R.damascena* was evaluated on human cervix cancer cell line (HeLa). This cell line was derived from cervical cancer cells taken from Henrietta Lacks in 1951. It has been one of the most widely studied cell lines in cervical cancer as the second most frequent malignant tumor in women worldwide (Verma and Hansch, 2006; Pisani et al., 1993).

Material and Methods

Reagents 3- (4,5-dimethylthiazol-2-yl) - 2,5-diphenyl tetrazolium (MTT) and Triton X-100 were purchased from Sigma. DMEM and FCS were purchased from Gibco.

Preparation of *Rosa damascena* extract *R. damascena* was collected from the School of Pharmacy and identified by a botanist. A voucher specimen was preserved in the Herbarium of the school of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran (Herbarium No: 254-1804-01). The plant extract was prepared as follows: Two hundred grams of the chopped, dried flowers of *R. damascena* was extracted for 3 days using 1500 ml 50% ethanol by digestion. The extract was reduced to dryness with a vacuum rotary evaporator.

Cell culture

HeLa Cells were incubated at 37°C in a humidified atmosphere (90%) containing 5% CO2. Malignant cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5% (v/v) fetal bovine serum, 100 units/ml penicillin, and 100 µg/ml streptomycin.

Cells were seeded overnight, and incubated with various concentrations of *R. damascena* extracts (30 to 1000 µg/mL) for 24, 48 and 72 h. For MTT assay, cells were seeded at 5000/well onto 96-well culture plates. For each concentration and time course study, there was a control sample which received an equal volume of medium. Paclitaxel (0.7 mmol) was used as positive control drug.

Cell viability

The cell viability was determined using a modified 3- (4, 5 -dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium (MTT) assay (Mosmann et al., 1983; Sharifi et al., 2005). Briefly, cells were seeded (5000/well) onto flat-bottomed 96-well culture plates and allowed to grow 24 h followed by treatment

with *R. damascena* extracts (30 to 1000 µg/mL) for 24 , 48 and 72 h. After removing the medium, cells were then labeled with MTT solution (5 mg/mL in PBS) for 4 h and the resulting formazan was solubilized with DMSO (100 µl). The absorption was measured at 570 nm (620 nm as a reference) in an ELISA reader.

Statistical analysis

All results were expressed as mean ± SEM. The significance of difference was evaluated with ANOVA and Bonferroni post-hoc test. A probability level of $p<0.05$ was considered statistically significant.

Results

Effect of *R. damascena* on cell viability

R.damascena decreased cell viability in HeLa cells, in a concentration - and time-dependent manner (Figure 1). This toxicity was consistent with morphologic changes including reduction in cell volume and rounding (data was not shown). Doses inducing 50% cell growth inhibition (IC50) against HeLa cells are presented in Table 1.

	24 hr	48hr	72hr
IC50	2135 µg.ml^{-1}	1540 µg.ml^{-1}	305.1 µg.ml^{-1}

Table 1.- IC50 of *R. damascena* ethanol extract against HeLa cell line. Cells were treated with different concentrations of R.damascena extract for 24, 48 and 72h. Viability was quantitated by MTT assay.
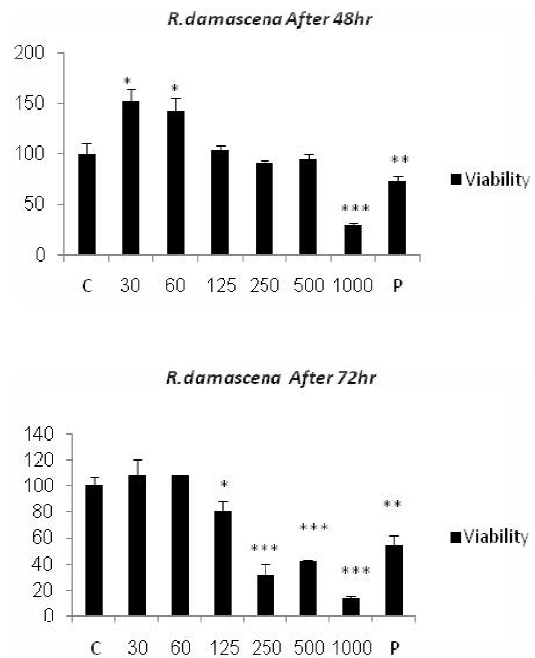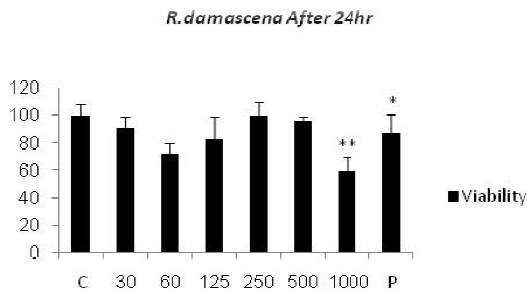

Figure 1. Effect of *R. damascena* ethanol extract on HeLa cell viability. Cells were treated with different concentrations of *R. damascena* extract for 24, 48 and 72h and Paclitaxel (P) as a posited control. Viability was measured by MTT assay. Results are shown as Mean ± SEM (n = 3). The asterisks are indicators of statistical differences obtained separately at different time points compared to their negative control shown in the figure as * p< 0.05, **p< 0.01 and ***p< 0.001.

Discussion

Cancer is a growing health problem in the world. Natural products have long been used to prevent and treat many diseases, including cancer, and thus they are good candidates for the development of anticancer drugs (Smith-Warner et al., 2000).

In the present study, the cytotoxic effects of *R. damascena* ethanol extract on HeLa cell line was investigated, which is the first experiment on HeLa cell line. Our data confirmed that *R. damascena* extract had cytotoxic activity against HeLa cell line which was consistent with previous studies indicating that *R. damascena* and its

ingredients possess antitumor and anticancerogenic activities (Zu et al., 2010). Taking into account the results of the current study, *R. damascena* could also be considered as a promising chemotherapeutic agent in cancer treatment.

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