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Resveratrol shows Anti-tumor Effects on AGS Gastric Cancer cell line by Inducing Mitochondrial Apoptotic Pathway

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Abstract

Background: Gastric cancer is one of the most common types of malignancies with a high mortality rate. Treatment approaches for this disease include surgery and chemotherapy, which are associated with high complications and low efficacy. Therefore, finding new therapeutic or adjuvant approaches is of great importance. This study aimed to investigate the effect of resveratrol (RSV) on the AGS gastric cancer cell line. Methods: After preparing and culturing AGS cells, they were treated with different concentrations of RSV for 48h. Then, cell viability and apoptosis rate were studied using MTT assay and flow cytometry technique, respectively. Moreover, after RNA extraction, cDNA synthesis and primer design, the expressions of *bax*, *bcl-2*, *casp3* and *p53* genes were studied using RT-PCR technique. Results: RSV showed cytotoxic effects on AGS cells in the MTT assay and decreased cell viability. In addition, cells exposed to 50 and 100 g/mL of RSV showed a significantly higher apoptosis rate compared to control cells. Finally, RSV overexpressed the *bax*, *p53*, and *casp3* genes and downregulated the *bcl-2* in AGS cells. Conclusion: Resveratrol is able to induce anticancer effects in gastric cancer by inducing apoptosis through the activation of mitochondrial apoptotic pathway and has the potential to be used as a therapeutic or adjuvant treatment option in gastric cancer patients.

Keywords: : Resveratrol, Gastric cancer, Apoptosis, Gene, Expression

1. Introduction

The growth and proliferation of cancer cells occur through mutations in genes that control cell division, which is associated with the increased growth of cancer cells and tumor development (1). One of the most common cancers in Iran is gastric cancer, which increased trend on this cancer has been reported in recent years and is the most common type of gastrointestinal malignancy (2). Given that most patients are diagnosed in advanced stages of the disease, therefore, the survival rate and effectiveness of treatment are greatly reduced (3). Treatment approaches for this disease include surgery and chemotherapy, which are associated with high side effects that severely reduce the quality of life of patients (4, 5). Furthermore, chemotherapy is associated with low efficacy and the development of drug resistance in cells (6), and the risk of metastasis after successful surgery is also extremely high (5). Therefore, it seems necessary to develop new therapeutic or adjuvant approaches in the treatment of gastric cancer. In the last three decades, plant-derived phytochemicals with anticancer and cancer-preventive properties

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have been the focus of researchers (7, 8), and one of these promising natural compounds is resveratrol (RSV). Resveratrol has been shown to have both cancer-preventive and anticancer effects (9). RSV is a stilbene polyphenol found in plants such as grapes and peanuts, and in vitro studies have shown its anticancer effects in a wide range of malignancies, including breast (10), cervical (11), thyroid (12), pancreatic (13), liver (14), and prostate (15) cancers. The action mechanism of RSV has been attributed to the induction of apoptosis and arrest of the cell division cycle in the G0-G1 phase (16, 17). In addition, RSV is able to sensitize cancer cells to chemotherapy and prevent drug resistance (18). These findings indicate that RSV has high potential in the treatment of various types of cancer. However, few studies have investigated the effects of RSV in gastric cancer. Therefore, this study aimed to investigate the anticancer effects of RSV on the AGS cell line with an emphasis on identifying its action mechanisms.

2. Materials and Methods

2.1. Study design, cell preparation, and culture

This descriptive-analytical study was conducted to investigate the anti-proliferative effects of RSV on the AGS gastric cancer cell line. For this purpose, AGS cells were purchased from the Pasteur Institute of Iran-Tehran. The cells were initially cultured in T25 flasks in DMEM medium supplemented with 10% FBS, penicillin (100U/mL), and streptomycin (100 g/mL) at 37°C and 5% CO_2. The cells were passaged once a week at a cell density of 70-80% and growth was maintained in the logarithmic phase.

2.2. Cell viability

After cell counting by Neobar slide, cell suspension at a concentration of (5×10^4 cells/mL) was poured into each well of a 96-well plate and incubated for 24 h. Then, after removing the culture medium, the cells were treated with different concentrations of RSV (12.5, 25, 50, and 100 µg/mL, Sigma, USA) for 48 h. In the next step, 5 mg/mL of MTT stock (Sigma, USA) was added to each well and incubated for 4 h. Then, after removing the MTT solution, 50 µL of DMSO was added to each well. After 15 minutes, the optical density (OD) was read at a wavelength of 570 nm by an ELISA reader.

2.3. Cell apoptosis

The apoptosis rate of cells was determined based on a recent study (19). For this purpose, after culturing cells in 96-well plates and treating them with different concentrations of RSV as described in the above section, the cells were washed with PBS and then trypsinized. Then, the separated cells were washed with 10% binding buffer solution (Ebioscience, USA) and centrifuged at 15,000 rpm for 15 min. 5 L of FITS solution (Ebioscience, USA) was added to each well and incubated for 15 min in the dark. Then, 5 L of annexin V and PI dyes (Sigma, USA) solutions were added and transferred to the flow cytometry device.

2.4. Gene expression

After treatment of cells, the RNA was extracted by RNA extraction kit (Yekta Tajhiz Azma, Iran) according the manufacturer's instructions. Next, the quality and quantity of extracted RNA were evaluated by agarose gel electrophoresis and NanoDrop techniques, respectively.

After ensuring the quantity and quality of extracted RNA, cDNA was synthesized using a commercial kit (Yekta Tajhiz Azma, Iran) based on the manufacturer's instructions.

The primers of genes studied in current study were designed by OLIGO 3 software and the accuracy of the sequences was examined by blasting the sequences in NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome). The sequences of forward and reverse primers are shown in the Table 1.

Table 1. The sequences of forward and reverse primer used for evaluating the expressions of *bax*, *bcl-2*, *casp3,* and *p53*

Genes	Forward [5'-3']	Reverse [5'-3']
p53	CCTCAGCATCTTATCCGAGTGG	TGGATGGTGGTACAGTCAGAGC
bax	TCAGGATGCGTCCACCAAGAAG	TGTGTCCACGGCGGCAATCATC
bcl-2	ATCGCCCTGTGGATGACTGAGT	GCCAGGAGAAATCAAACAGAGGC
casp3	GGAAGCGAATCAATGGACTCTGG	GCATCGACATCTGTACCAGACC
GAPDH	GTGAACCATGAGAAGTATGACAAC	CATGAGTCCTTCCACGATACC

The temperature-time program of the RT-PCR device were 1 cycle of 95°C for 5 min, 40 cycles of 95°C for 5 s, 65-62°C for 20 s, and 72°C for 30 s.

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2.5. Statistical analysis

The gene expression data were analyzed by the 2^{-CT} method and the cell viability and apoptosis were analyzed by ANOVA procedure at a significant level of P<0.05 using Tukey's multiple range test for mean comparisons.

3. Results

3.1. Cell viability and apoptosis

We studied the cytotoxic effects of RSV on the gastric cancer cell line AGS using the MTT assay, and the results indicated a concentration-dependent cytotoxic effect of this natural compound. 50 and 100 g/mL of RSV inhibited the AGS cell viability by approximately 45 and 70%, respectively, which showed significant differences from control cells (Figure 1a). The concentration of 100 g/mL of RSV had the strongest inhibitory effects on the survival of AGS cells, such that the decrease in the viability of cells exposed to RSV at this concentration was significantly different from that of cells exposed to 50 g/mL of RSV (P = 0.046). The cytotoxic effects of RSV were attributed to the induction of apoptosis in AGS cells, and 50 and 100 g/mL RSV showed the highest percentage of cell apoptosis. It is worth noting that the percentage of cell apoptosis in AGS cells exposed to 100 g/mL RSV was significantly higher than in AGS cells treated with 50 g/mL of this natural compound (P=0.002, Figure 1b).

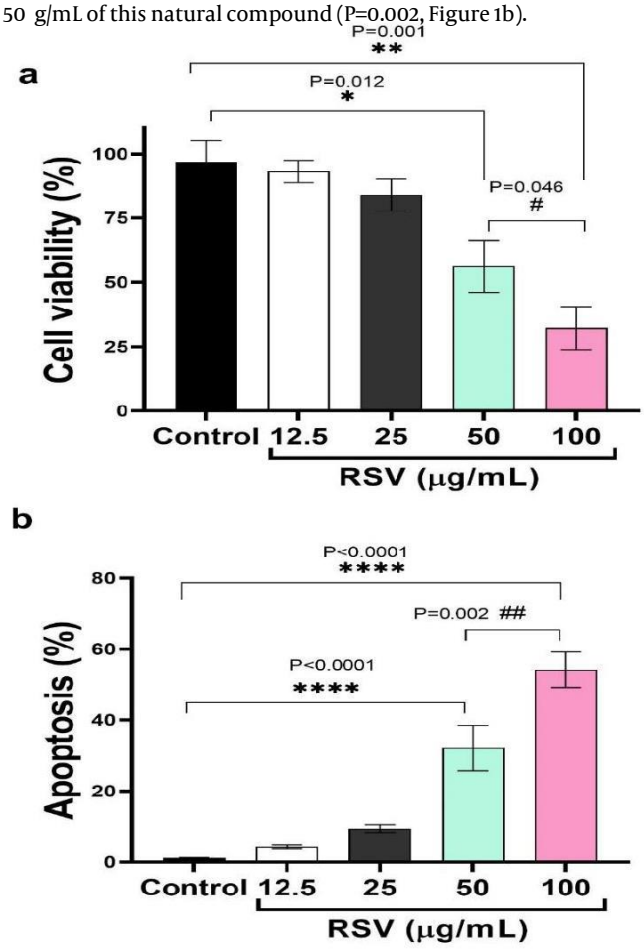

Figure 1. The impacts of different concentrations of resveratrol (RSV) on AGS cell line viability (b) and apoptosis rate (b). The cells were treated with RSV for 48h and cell viability and apoptosis were measured using MTT assay and flowcytometry technique (n=3).

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3.2. Gene expression

50 and 100 g/mL of RSV overexpressed *bax* in AGS cells, but downregulated *bcl-2* (Figure 2). AGS cells exposed to high concentrations of RSV (50 and 100 g/mL) showed significant differences in the expression of both *bax* and *bcl-2* genes compared to untreated cells (control), with *bax* expression being upregulated and *bcl-2* expression downregulated. This suggests that RSV is able to induce apoptosis in AGS cells by affecting the expressions of *bax* and *bcl-2*.

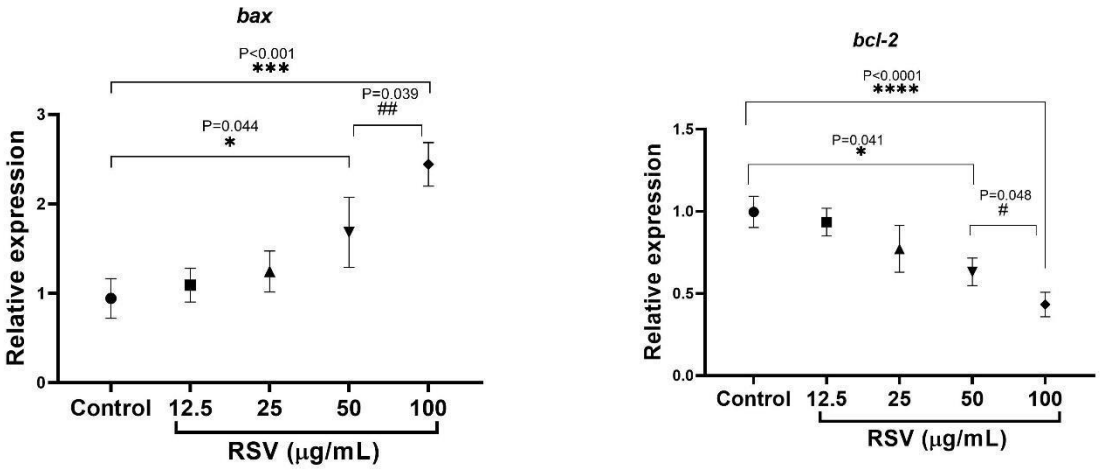

Figure 2. The effects of different concentrations of resveratrol (RSV) on the expression of *bax* and *bcl-2* genes in AGS cell line (n=3). The cells treated with RSV for 48h and the expressions of aforementioned genes were studied by RT-PCR.

Furthermore, 50 and 100 g/mL RSV significantly overexpressed *casp3* gene in AGS cell line compared with untreated ones; however, there were no significant differences in the expression of *casp3* gene between cells treated with 50 and 100 g/mL RSV (P=0.145, Figure 3). Finally, 100 g/mL RVS could significantly upregulated *p53* gene expression compared with control cell (P=0.14).

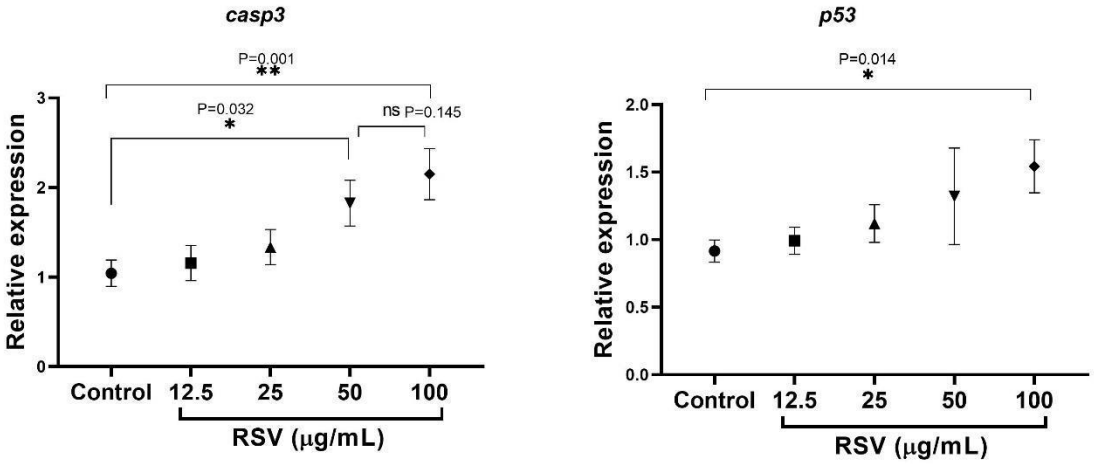

Figure 3. The effects of different concentrations of resveratrol (RSV) on the expression of *casp3* and *p53* genes in AGS cell line (n=3). The cells treated with RSV for 48h and the expressions of aforementioned genes were studied by RT-PCR.

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4. Discussion

The findings of the present study indicated the anticancer effects of resveratrol on the AGS gastric cancer cell line, and the cytotoxic effects of this natural compound were confirmed in the MTT assay. The death of cancer cells by resveratrol was attributed to the induction of apoptosis, and it was found that resveratrol induces apoptosis in the AGS gastric cancer cell line by overexpressing *bax*, *casp3,* and *p53* genes and downregulating *bcl-2*.

Resveratrol has a wide range of pharmacological effects such as cardioprotective (20), neuroprotective (21), anti-diabetic (22), anti-inflammatory (23), and antioxidant (24), and recently the anticancer effects of RSV have attracted the attention of researchers. For example, this natural compound has shown anticancer effects in a wide range of malignancies such as thyroid (25), oral (26), colorectal (27), lung (28), and prostate (29) cancers, and the present study showed that RSV also has anticancer effects in gastric cancer, which is similar to the findings of the aforementioned studies. The mechanisms of action of the anticancer effects of RSV have been attributed to the arrest of the mitotic cell division cycle, inhibition of EMT (anti-metastatic effects), and induction of apoptosis (30). It has been shown that exposure of cancer cells to RSV induces apoptosis through the downregulation of NF- B and the upregulation of *casp3* and *bax*, which is accompanied by the downregulation of *bcl-2* (31). In the present study, it was also shown that resveratrol (100 g/ml) was able to induce apoptosis by up-regulating *bax*, *bcl-2*, and *p53* and down-regulating *bcl-2*, which is similar to the findings of other studies. This finding suggests that RSV is able to activate the mitochondrial apoptotic pathway in cancer cells, which is accompanied by a decrease in cell viability. In a study, administration of curcumin and RSV reduced the proliferation of gastric cancer tumors in a rat model through up-regulating *p53* (32), which is similar to the findings of the present study. Therefore, it appears that RSV induces anticancer effects in the AGS cell line by disrupting mitochondrial function.

5. Conclusion

Overall, it is concluded that RSV has anticancer effects in gastric cancer and its action mechanisms are the disruption of mitochondrial function and induction of apoptosis, which is associated with upregulation of *bax*, *p53*, and *casp3* and downregulation of *bcl-2*.

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