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RESEARCH ARTICLE

Red grape seed extract and its compound resveratrol exert cytotoxic effect to various human cancer lines

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ABSTRACT

Modern medicinal agents currently available for treatment of cancers are very expensive, toxic, and less effective in treating some of the disease. Thus, one must investigate further in detail the agents derived from natural sources, such as grape seed, for the prevention and treatment of cancer and disease. In recent years interest of researchers has focused on grape seed and nowadays scientists have used extracts of grape seed to treat different health problems including cancer. We examined the cytotoxic effect of red grape seed extract (GSE) and its main compound resveratrol (RES) on different human cancer cell lines representing various solid tumors and hematological malignancies at the same time. Red GSE was prepared by using 1, 1, 1, 2-Tetrafluoroethane extraction method. Cytotoxicity of the extract and RES was evaluated by using trypan blue dye exclusion method and MTT assay. The results of our study show that GSE and RES have cytotoxic activities in varying degree in several cancer cell lines. There has not been any study evaluating the GES and RES in the same cell lines and in the same conditions. But, it is still needed to have more pre-clinical and laboratory studies to validate the usefulness of these agents either alone or in combination with existing therapy.

Key Words: Grape seed extract, resveratrol, cancer cell line, cytotoxicity

ÖZET

Kırmızı üzüm çekirdeği özütü ve bileşiklerinden resveratrol değişik insan kanser hücre dizilerinde sitotoksik etki göstermektedir

Günümüzde kanser tedavisinde kullandığımız birçok ilaç oldukça pahalı, toksik ve etkinlikleri de sınırlıdır. Bu nedenle üzüm çekirdeği gibi doğal ürünlerden kaynaklanan ajanların kanser ve diğer hastalıklardan korunma ve tedavisine yönelik birçok çalışma yapılmaktadır. Son yıllarda üzüm çekirdeği üzerine odaklanılmış ve özütü kanser de dahil birçok hastalıkta kullanılmaya başlanmıştır. Biz farklı kanser hücre dizilerinde kırmızı üzüm çekirdeği özütü ve bileşiminde bulunan resveratrolün aynı zamanda sitotoksik etkinliklerinin olup olmadığını değerlendirdik. Kırmızı üzüm çekirdeği özütü 1, 1, 1, 2-Tetrafluoroethane extraction metodu uygulanarak elde edildi. Sitotoksisitenin değerlendirilmesi için trypan blue dye exclusion metodu ve MTT yöntemi kullanıldı. Sonuçlarımız bize üzüm çekirdeği özütü ve resveratrolün değişik kanser hücre dizilerinde sitotoksik etki gösterdiğini ortaya koydu. Hem üzüm çekirdeği özütü hem de resveratrolün sitotoksisitesini aynı koşullarda ve eş zamanlı olarak gösteren çalışma mevcut değildir. Ancak, bu ajanların gerek tek başlarına gerekse mevcut tedavilerle kombine olarak kullanılmalarının onaylanması için daha fazla preklinik ve laboratuvar çalışmasına ihtiyaç vardır.

Anahtar Sözcükler: Üzüm çekirdeği özütü, resveratrol, kanser hücre dizisi, sitotoksisite

INTRODUCTION

Dietary agents consist of a wide variety of biologically active compounds that are ubiquitous in plants, many of which have been used in traditional medicines from Hippocrates. Researches over the last decade have shown that several micronutrients in fruits and vegetables reduce cancer. Grapes, the succulent fruits of the grapevine (Vitis vinifera L.), are rich in polyphenolic compounds with biological activity. The medicinal and nutritional value of grapes has been known for thousands of years. The ancient Egyptians praised the medicinal qualities of grapes [1]. In recent years, interest has focused on grape seed and nowadays scientists use standardized extracts of grape seed to treat different health problems, including cancer. Studies in laboratories and animals lend some support to these uses. An extract of the grape seed (GSE) is of particular clinical interest due to its high content of polyphenolic compounds such as resveratrol.

Resveratrol (RES) is a natural polyphenol present in red wines and other constituents of the human diet [2]. RES concentrations in wine are associated with the fermentation time in contact with grape skins since RES is produced by the skin and seeds. This also explains the low concentrations in white wine. RES has been an important constituent of Japanese and Chinese folk medicine. Indirect evidence suggests that the presence of RES in wine may explain the reduced risk of coronary heart disease associated with moderate wine consumption. This effect has been ascribed to anti-aggregate, anti-inflammatory and anti-oxidant activity of RES. Traditional Chinese medicine and more recent epidemiological studies have strongly supported the idea that RES may act as a cancer chemo preventive compound [3,4]. RES affects different intracellular signaling pathways and causes a complete and reversible cell cycle arrest in the S and G2 phases [5,6]. RES has been reported to inhibit cell growth and to induce apoptosis in various solid and hematological cancer cell lines [6-12].

We examined the cytotoxic effects of red GSE and its compound RES at various concentrations in nine human cancer cell lines as a model of tumors.

MATERIALS and METHODS

Chemicals

RES was purchased from Sigma Aldrich, Germany. Penicillin-streptomycin, L-glutamine, RPMI-1640, and fetal bovine serum (FBS) were obtained from Biochrome KG, Berlin,

Germany. GSE was extracted from red grapes grown in the Ege region of Turkey by using 1,1,1,2-Tetrafluoroethane extraction method by Yasar Hisil in the Food Science Department, Engineering Faculty, Ege University. A stock solution of RES was prepared in dimethylsulfoxide (DMSO) at a concentration of 100 mmol/L and then diluted in cell culture medium. The DMSO concentration did not exceed 0.1% in any experiment.

Cell Lines and Cultures

The HL-60 human promyelocytic leukemia cell line, K-562 human eryhtroleukemia cell line, CCRF-CEM human acute lymphoblastic leukemia cell line, ARH-77 and RPMI-8226 human multiple myeloma cell lines, G-361 [CRL-1424] human malignant melanoma cell line, M-DAH human ovarian carcinoma cell line, MCF-7 human breast cancer cell line and DU-145 human prostate carcinoma cell lines were maintained in RPMI-1640 medium supplemented with 10% heat-inactivated FBS (Biochrome KG, Berlin, Germany), antibiotics (100 units/mL penicillin and 100 µg/mL streptomycin) and 2 mM L-glutamine under an atmosphere of 95% air and 5% CO_2 at 37°C.

Analysis of Cell Viability and Cytotoxicity

Cell viability was determined by trypan blue exclusion. All cells were incubated with various dilutions of GSE and various concentrations of RES for 96 hours.

The effect of RES on cell proliferation was measured using an MTT-based assay as described previously. Briefly, the cells (5.000/well) were incubated in a 96-well plate in the presence of various concentrations of GSE and RES in a final volume of 0.2 mL for 24-96 hours. Every day, 0.025 mL of MTT solution was added to each well and then incubated for four hours. Afterwards, the supernatant was removed from each well. The colored formazan crystal produced from MTT was dissolved in 1N isopropyl alcohol and the absorbance value (A) proportional to the degree of cell viability determined at 540 nm was read using an automatic multiwell spectrophotometer (Bio-Rad-Coda, Richmond, CA). The negative control well was used for zeroing absorbance. The percentage of cytotoxicity was calculated using the background-corrected absorbance as follows:

Cytotoxicity (%) = (1-A of experiment well/A of positive control well) x 100.

RESULTS

We examined the effect of GSE and RES at various dilutions and concentrations on the cytotoxicity in nine human cancer cell lines. Cancer cell lines were cultured in complete medium in the absence (control) or presence of various dilutions of GSE (1/1000-1/100.000) and various concentrations of RES (1 nM-100 µM) for 96 hours. Untreated cells (control) were considered as the baseline (100%) for the analysis.

Using trypan blue dye exclusion test and MTT assay, GSE yielded an IC_{50} value of 1/10000 in HL-60, K-562, CCRF-CEM, M-DAH and MCF-7 cell lines and 1/5000 in ARH-77, RPMI-8226, G-361 [CRL-1424] and DU-145 cell lines after incubation.

After various concentrations of RES treatment of cell lines, IC_{50} values were determined as 0.1 µM in CCRF-CEM, 1 µM in M-DAH, MCF-7, and DU-145, 5 µM in HL-60, 10 µM in ARH-77 and RPMI-8226, and 100 µM in K-562 and G-361 [CRL-1424] cell lines by using trypan blue dye exclusion test and MTT assay. The values of IC_{50} of GSE and RES were compiled in Table 1. Figure 1 represents the results of all cytotoxicity experiments in each cell line.

The cytotoxic effects of GSE and RES on the viability of all cancer cell lines were also determined by MTT assay and the doses obtained in MTT assay are illustrated in Table 1. Only the cytotoxicity data obtained by trypan blue in GSE and by MTT in RES are presented in Figure 1, but other results were also similar.

DISCUSSION

GSE is derived from seeds of purple or red grapes, the same kind that are pressed to pro-

Table 1. The values of IC_{50} of Grape Seed Extract (GSE) and Resveratrol (RES)

Cell lines	GSE (value of IC_{50}) (dilution)	RES (value of IC_{50}) (μM)
HL-60	1/10000	5
K-562	1/10000	100
CCRF-CEM	1/10000	0.1
ARH-77	1/5000	10
RPMI-8226	1/5000	10
G-361 [CRL-1424]	1/5000	100
M-DAH	1/10000	1
MCF-7	1/10000	1
DU-145	1/5000	1

duce red wine. GSE has plenty of high RES content. RES is a polyphenolic compound that has demonstrated anti-oxidant, anti-aging, anti-inflammatory, anti-cancer, and anti-atherogenic, etc., properties [2].

Epidemiological data indicate that vegetables and fruits containing chemopreventive agents could have protective effect against cancer. However, due to lack of sufficient preclinical and clinical studies, it has been difficult to generalize this hypothesis. On the other hand, the major advantage of chemoprevention could be defined as lesser toxicity compared to standard medicinals. Another advantage of chemoprevention has been reported recently as the improvement of the therapeutic response of other treatment modalities in case of combinational use, such as chemo-or radiotherapy in cell culture studies [13].

A well-known theory, which is called "The French Paradox", is comprised of some of the beneficial effects of the Mediterranean Style Diet containing flavonoids found in red wine. The belief is that drinking wine protects from development of some diseases such as atherosclerotic heart disease and provides for the role of a potent chemopreventive compound in blocking the initiation of inflammation and oncogenesis [14].

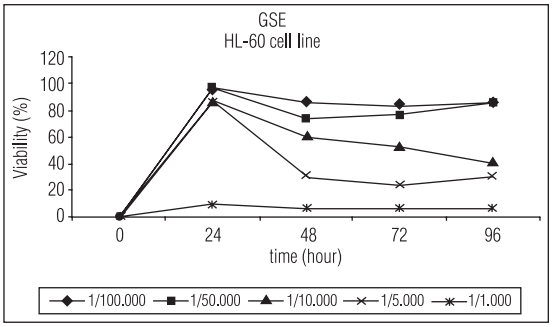
As shown in Table 1, RES induced dose-dependent decrease in viable cell count, compared with cells incubated in medium alone. The data also indicated that RSE sensitivities were different while GSE sensitivities were similar in all of them. IC_{50} dose of GSE was found to be similar especially in leukemic cell lines (HL-60, K-562 and CCRF-CEM), but the sensitivity of these cell lines showed different values with respect to RES. CCRF-CEM cell line was highly sensitive to RES, whereas the K-562 cell line was more resistant to RES compared with other leukemia cell lines. Numerous studies exist that have utilized a wide range of concentrations of RES, suggesting that its biological effects may vary depending on cell types.

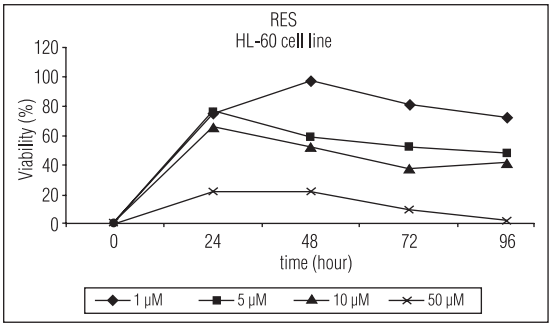
Extensive data in human cell cultures indicate that RES can modulate multiple pathways involved in cell growth, apoptosis, and inflammation. The anti-carcinogenic effects of RES appear to be closely associated with its antioxidant activity, and it has been shown to inhibit cyclooxygenase, hydroperoxidase, protein kinase C, Bcl-2 phosphorylation, Akt, focal adhesion kinase, NFB, and cell cycle regulators. RES has the capacity to interact with multiple molecular targets and affects different intracellular signal pathways as MAPK pathways. For example, tumor necrosis factor (TNF)-α-induced JNK and MEK (MAPK kinase) activation were inhibited in U937 cells by pretreatment with RES. RES suppresses TNF-α induced activation of nuclear transcription factors NF-kappa B, activator protein-1, and apoptosis. In HeLa cell line, pretreatment with RES inhibited phosphorylation of ERK2 and JNK [3,10,15-18].

Ragione et al. [5] documented the possible RES IC_{50} value in HL-60 cell line as 10 μM. RES inhibited cell growth at a concentration as low as 30 μM while at higher concentrations, such as 50-100 μM, it appeared to be toxic. In our study, IC_{50} value was determined as 5 μM. This value is similar to the dose found by Ragione et al. They demonstrated by flow cytometry that RES blocks cell proliferation at the S/G2 boundary [5]. The effect of RES on the cell cycle seems to focus on the S-

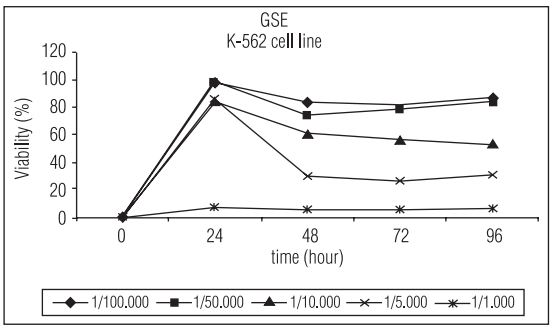
Figure 1. The demonstration of cytotoxic effect of GSE by trypan blue method and of RES with MTT assay in different cell lines.

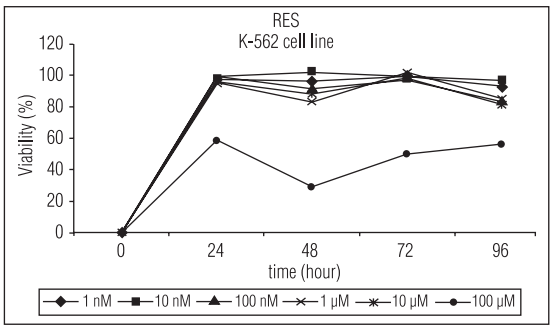
a. HL-60 Cell Line



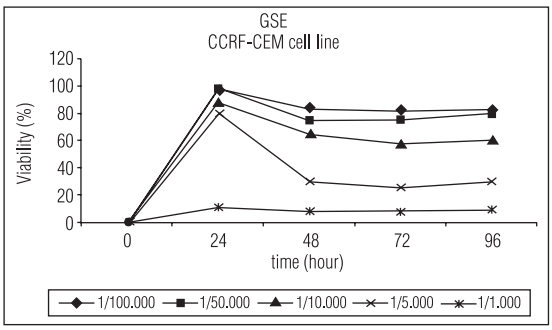


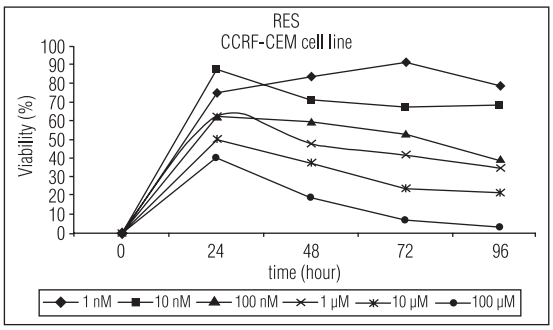
b. K-562 Cell Line



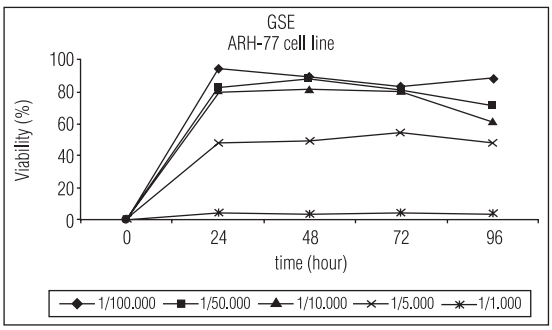


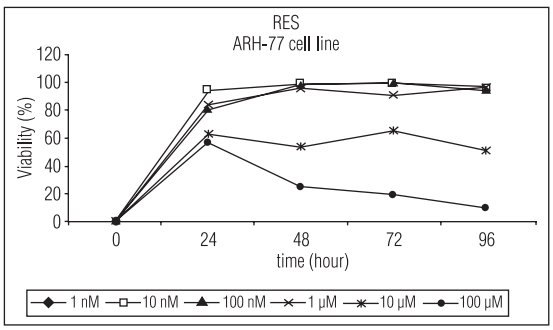
c. CCRF-CEM Cell Line



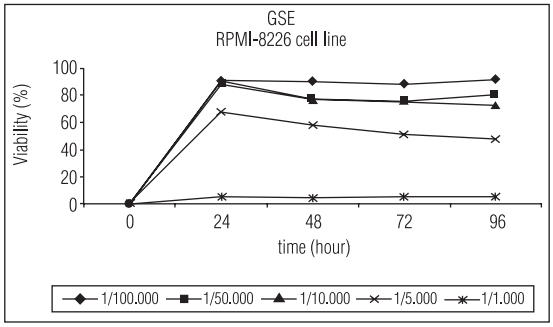


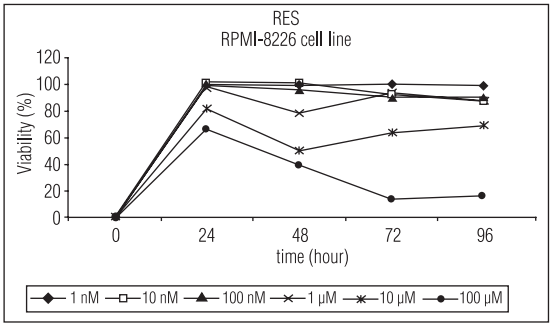
d. ARH-77 Cell Line



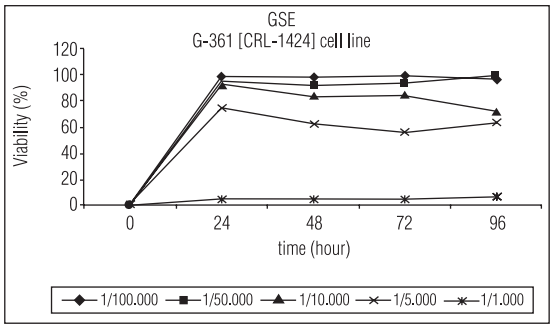


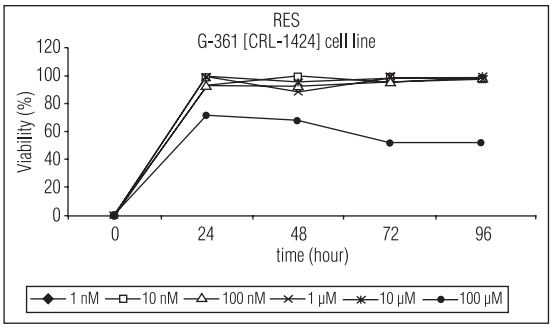
e. RPMI-8226 Cell Line



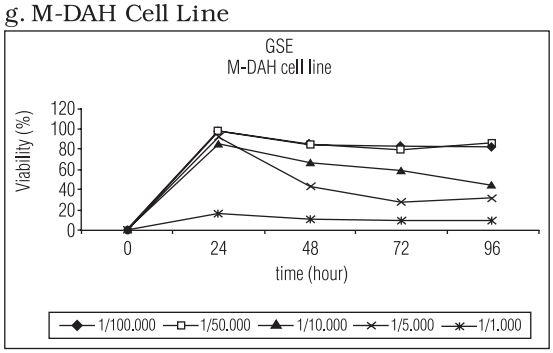


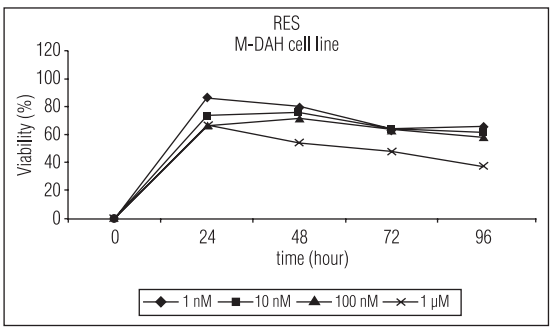
f. G-361 [CRL-1424] Cell Line



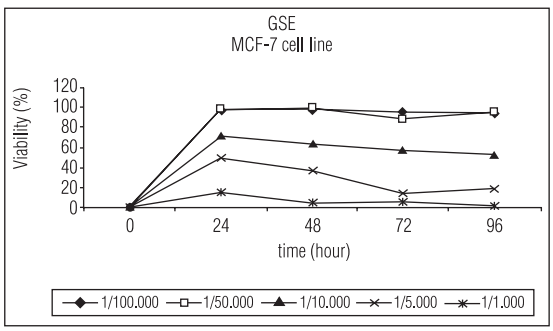


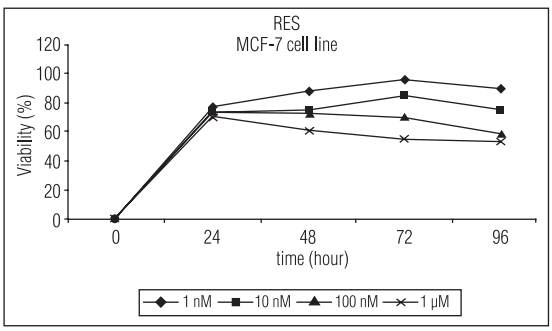
g. M-DAH Cell Line



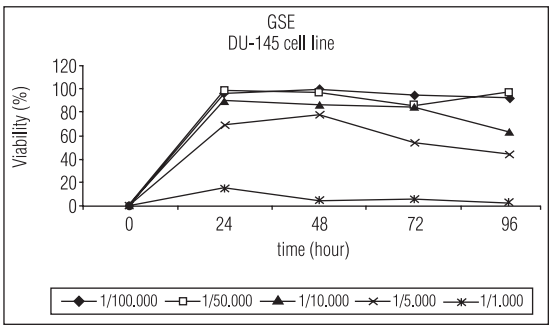


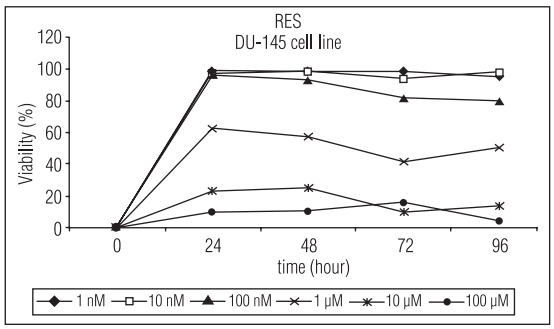
h. MCF-7 Cell Line





i. DU-145 Cell Line





phase. A cell cycle arrest in the S-phase has been reported for different cell lines [19-21].

RES mostly induces apoptosis by caspases systems. RES was shown to activate pro-apoptotic caspases-6, -3, -9 and was partly independent of caspase-8 activation. It also leads to an induction of p53 and bax, and to an inhibition of bcl-2. RES upregulation of the bax protein correlated with an activation of caspase-3 and caspase-9 in a dose-dependent manner in human prostate carcinoma cell lines (DU-145) [3,22].

The combined effects of RES were tested with Ara-C and showed synergistic growth inhibition and apoptosis induction in HL-60 cells [21]. Gautam et al. reported that leukemia cells were more sensitive than normal hematopoietic cells to the antiproliferative effects of RES, an event that may help ex vivo leukemia cell purge from precursor cell preparations before auto-transplantation [23].

RES has been reported to have a cytotoxic effect in various human cancer cell lines [24-28]. The results of our study show that GSE and RES have cytotoxic activities in various cancer cell lines. There has not been any study evaluating GSE and RES in the same cell lines and in the same conditions. According to our knowledge, our study is the first showing the anti-cancer effect of both GSE and RES at the same time and in the same cell line under the same conditions. Thus, this data could possibly provide the rationale for use of GSE in a chemo-preventive manner in the future.

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