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The Oxidative and Cytotoxic Effects of Resveratrol Compared With Cisplatin in the LNCap Prostate Cancer Cell Line

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Abstract

Introduction: Prostate cancer is one of the most common diseases worldwide, and many efforts are made to treat this disease; however, these efforts seem to be futile. Accordingly, this study tends to compare the cytotoxic and oxidative (as shown by the production of reactive oxygen species (ROS)) effects of resveratrol in comparison with cisplatin in a prostate cancer cell line (LNCap).

Methods: After treatment of LNCap cells with the concentrations of 1, 10, 100, 500, and 1000 µg/mL of resveratrol, and 1, 5, 10, and 50 µg/mL of cisplatin, the viability percentage of cells was analyzed using MTT and spectrophotometry. Also, ROS production in this cell line at the IC_{50} concentrations of cisplatin and resveratrol was analyzed using flow cytometry.

Results: The results of the MTT test showed that cisplatin and resveratrol could both reduce cell viability at high concentrations because of increased cytotoxicity. Measurement of ROS production showed that cisplatin and resveratrol increased ROS levels compared to control cells.

Conclusion: The results obtained from this study showed that resveratrol, as a plant derivative, had anticancer and antioxidant properties against the LNCap prostate cancer cell line. Because of the side effects of cisplatin, it can be replaced by resveratrol as a good candidate to be used in chemotherapy.

Keywords: Prostate cancer, Resveratrol, Cisplatin, Reactive oxygen species

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Introduction

Prostate cancer is one of the most underlying causes of mortality across the world. The disease is the second common cancer and the fifth cause of mortality in men, and the third fatal cancer in all countries.[1] Prostate cancer, same as other cancers, can be because of mutation in important regulative genes, which is created mostly by a carcinogenic or mutagenic factor or because of dysfunction in DNA repair.[2] Early diagnosis of the disease by serum examination for prostate-specific antigen (PSA), surgical operation, and radiotherapy, can decrease the mortality caused by prostate cancer considerably. However, still, there is no effective treatment for the late stages of the disease.[3] Combination chemotherapy with cisplatin is at the heart of treatment for many types of cancer. Although responses to cisplatin are initially high; many cancer patients eventually relapse with cisplatin-resistant disease. Therefore, drug resistance was observed in many patients who relapsed after treatment with cisplatin. Proposed mechanisms for cisplatin resistance include alterations in cellular uptake.

Biotransformation and detoxification in the liver enhances DNA repair and anti-apoptotic mechanism. Traditionally, using medicinal plants is common in different societies. In Iran, the majority of people use medicinal plants to prevent and treat many diseases. Medicinal plants are valuable natural resources, which have gained the attention of the developed countries and are being used as raw materials to make riskless medicines for human beings. In this field, Iran is one of the richest sources of medicinal plants with a high diversity of habitat conditions for these plant species.[5,6]

The proposed mechanisms proving the protective effect of medicinal plants include deceased oxidative/nitrative stress; decreased lipid peroxidation; prevention of DNA fragmentation and oxidative damage; the decreased activity of microglia and astrocytes; inhibition of apoptotic protein expression; increased mitochondria gene expression; decreased eicosanoids including leukotrienes, prostaglandins, and thromboxane; increased expression of anti-apoptosis proteins, and decreased expression of inflammatory mediators.[7-9] Various studies have revealed that different plants possess antioxidant effects.[10,11] Nowadays, natural antioxidants extracted from plants and additives are being evaluated in a wide range, and it is believed that natural antioxidants leave the least side effects.[12]

Resveratrol (3, 4', 5-trihydroxy-trans-stilbene) is a phytoalexin, which is created in many plant species in response to mechanical damage, fungal infection, and ultraviolet ray.[13] Anticancer properties of resveratrol are originated in its anti-oxidative, anti-inflammatory, anti-mutation, and anti-proliferative properties.[14,15] Resveratrol inhibits the proliferation of cancer cells, begins apoptosis, and adapts the function of a wide range of pro-apoptosis and anti-apoptosis factors to differentiate cancer cells, reduce inflammatory reactions, and to neutralize free radicals.[14,16-18] Because of a wide range of biological activities and using signaling path adjustment, resveratrol is capable to block cancer progression in all stages including initiation, promotion, and progression.[19-21] Resveratrol is an organic cleaner for hydroxyls, superoxide, and the free radicals that are produced by metals and enzymes. Besides, lipid peroxidation in the cell membrane, and DNA damage by reactive oxygen species (ROS) are prevented by resveratrol.[22] The material with its antioxidant and antitumor property inhibits cell proliferation, angiogenesis, and induction of cancer cell susceptibility to chemotherapy medicines.[23]

Anticancer properties of resveratrol have been confirmed on several cell lines; although its effect on LNCap cells is not examined to date. Accordingly, the purpose of this study was the evaluation of cytotoxicity of resveratrol and its effect on ROS production level in LNCap cell line compared to cisplatin.

Materials and Methods

Preparation and Cultivation of Cells

The prostate cancer LNCap cell line was prepared from the Pasteur Institute Research Center, Tehran. After transferring the cells to the laboratory, they were cultured in the DMEM medium containing 10% of enriched fasting blood sugar (FBS) under the temperature of 37°C and 5% of CO_2. 1×10^6 LNCap cells were cultured in two 60 well plates and were incubated for 24 hours so that each of them could be used to examine the viability of cells and measurement of ROS level.

Preparing Resveratrol

To prepare resveratrol ($C_{14}H_{12}O_3$), 100-mg resveratrol capsules (SOLGAR, Turkey) were purchased. To provide resveratrol stock concentration, a 100-mg capsule was solved in 2% DMSO and the standard resveratrol stock solution was prepared. Different concentrations of resveratrol (1, 10, 100, 500 and 1000 µg/mL) were prepared from the standard stock solution.

Cisplatin Preparation

The cisplatin powder (Sigma, Germany) was solved in an appropriate proportion of normal saline, and a standard stock solution was prepared. Concentrations of 1, 5, 10, 20 and 50 µg/mL from the standard stock solution were prepared.

Treatment of Cells

After 24 hours, one of the 60 well cell culture plates was taken out of the incubator, and the supernatant of each well of the plate was removed. Then, 10 wells of the 60 well plate were considered as the control group (untreated prostate cancer cells), and the other 50 wells were divided into two 25-member groups for various concentrations of cisplatin and resveratrol. After adding various concentrations of cisplatin (1, 5, 10, 20 and 50 µg/mL) and resveratrol (1, 10, 100, 500 and 1000 µg/mL) to the wells, the plate was placed in the incubator for 24 hours.

Cell Viability Determination

In this study, the cell viability was evaluated by using MTT (dimethyl thiazole-2 and 5-diphenyltetrazolium bromide) method. After removing the supernatant of plate wells, 500 µL of MTT was mixed with 4500 µL of culture medium without serum, and 100 µL of the solution was added to all wells of the plate and was incubated for 3-5 hours. Afterward, the plate was taken out of the incubator, and the supernatant of each well was removed. Then, 100 µL of DMSO (dimethyl sulfoxide) was added to each well. After 10 minutes, their optical absorption was measured at wavelength of 500-600 nm by a spectrophotometer. In this study, PBS optical density was considered as blank absorbance, and its value was subtracted in the estimations. After determining the optical density of treated prostate cancer cells, the cell viability was measured as follows:

$$\text{survival} = \frac{100 \times \text{sample absobance}}{\text{control absorbance}}$$

Then, according to the data obtained from the formula, the IC_{50} value (the concentration, in which half of the cells are survived and half of them are destroyed) was measured separately for cisplatin and resveratrol.[24,25]

Reactive Oxygen Species Production Measurement

After 24 hours, the second plate was taken out of the

incubator, and the supernatant of plate wells was removed. One milliliter of cisplatin and resveratrol was added to relevant wells with IC50 concentration, and 1 mL culture medium was added to control wells. Then, the plate was incubated for 24 hours. Afterward, the content of each well was poured separately in 9 Falcon tubes with a capacity of 15 mL (3 cisplatins, 3 resveratrol, and 3 control). By adding trypsin to the plate wells, the cells were removed and transferred to the relevant Falcons tubes. The tubes were centrifuged for 5 minutes with a speed of 2000 rpm. After removing the supernatant, the deposit was washed by 1000 µL of PBS. Measurement of produced ROS was done by using oxygen free radical detection laboratory kit (Abcam, UK). After adding 10µg DCFH-DA to the plates, they were incubated in 5% CO_2 under the temperature of 37°C for 15 minutes. DCFH-DA enters the cell in the cell membrane by deacetylated esterase. When the DCFH-DA was oxidized by ROS, a green fluorescent was created by that and was tracked by a flow cytometer.[26,27]

Data Analysis

The data analysis was done using SPSS version 22. The distribution of data was checked using Kolmogorov-Smirnov test that showed normal distribution. ANOVA, and Tukey post hoc test were used for statistical analysis. The $P<0.05$ level was considered as the significance level.

Results

Viability of LNCap Cells Treated with Cisplatin

The results obtained from the treatment of LNCap cells with 1, 5, 10, 20, and 50 µg/mL of cisplatin are illustrated in Figure 1. According to the diagram in Figure 1, there is a significant difference ($P<0.05$) between the groups treated with different concentrations of cisplatin and the control group. In high cisplatin concentrations (50 µg/mL), the viability of cancer cells encountered more than 50% reduction. Besides, there was a significant difference ($P<0.05$) between the viability of cells under various concentrations of cisplatin (except for 1 and 5 µg/mL).

Using Tukey post hoc test, the mean survival percentage of prostate cancer cells treated at different concentrations of cisplatin after 5 replications (1, 5, 10, 20 and 50 µg/mL) were compared with each other and also with the control group. The results are as follows:

At the concentrations of 1, 5, 10, 20 and 50 µg/mL cisplatin, the mean cell viability was 85.19, 78.12, 57.77, 37.22 and 11.49, respectively. Tukey post hoc test shows that there is a significant difference between the mean survival percentage at different concentrations of cisplatin ($P<0.05$) and the control sample (100% live). In fact, at the highest concentrations of cisplatin (50 µg/mL), the lowest mean percentage of prostate cancer cell survival is observed.

The Viability of LNCap Cells Treated With Resveratrol

The results obtained from treatment of prostate cancer cells by 1, 10, 100, 500, and 1000 µg/mL concentrations of resveratrol are illustrated in Figure 2. According to Figure 2, except for a concentration of 1 µg/mL, a significant difference was observed between the treated groups with other concentrations of resveratrol and the control group in terms of viability percentage ($P<0.05$). The viability between various concentrations of resveratrol showed a significant difference ($P<0.05$) (except for concentrations of 500 and 1000 µg/mL). Using Tukey post hoc test, a comparison was made between the mean survival percentage of cells treated with certain concentrations of resveratrol (1, 10, 100, 500, 1000 g/mL) after 5 replications for each concentration.

At concentrations of 1, 10, 100, 500, 1000 µg/mL of cisplatin, the mean cell viability was 93.66, 73.85, 57.88, 42.63 and 33.28, respectively. Tukey post hoc test showed that there was a significant difference between the mean

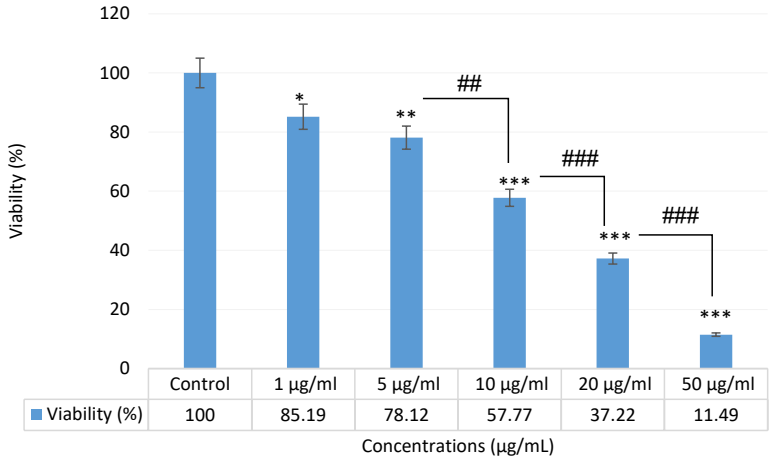

Figure 1. The Diagram of Viability Percentage of LNCap Cells in Presence of Various Concentrations of Cisplatin (* Significant difference with the control group at the level of $P<0.05$; ** Significant difference with the control group at the level of $P<0.01$; *** Significant difference with the control group at the level of $P<0.001$; ## Significant difference with the control group at the level of $P<0.001$)

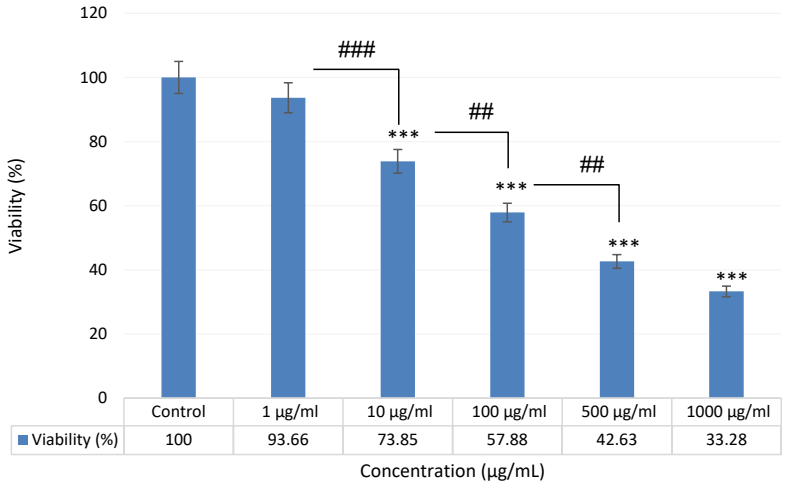

Figure 2. The diagram of the viability of LNCap cells in presence of various concentrations of resveratrol (*** Significant difference with the control group at the level of $P<0.001$; the significant difference with the control group at the level of $P<0.01$; ### Significant difference with the control group at the level of $P<0.001$)

survival percentage due to different concentrations of resveratrol ($P<0.05$) and with the control sample except for the concentration of 1 µg/mL which was not significantly different from the control group and Concentrations of 500 and 1000 µg/mL were not significantly different.

IC50 of Resveratrol and Cisplatin for LNCap Cells

The IC_{50} concentration for resveratrol and cisplatin is estimated respectively at 205.6 and 12.54 µg/mL according to Figure 3.

ROS Production Level in LNCap Cells

The histogram of the control group is divided into two gating areas as constant limits. The right gating determines the DCFH+percentage or the ROS percentage, and the left gating determined the DCFH- or lack of ROS production. The results obtained from 3 iterations illustrated as a histogram in control, cisplatin, and resveratrol groups are shown in 3 diagrams in Figure 4.

As illustrated in Figure 5, there is a significant difference between the control and cisplatin groups in terms of ROS production level ($P<0.05$). Besides, the diagram of the ORS production level in the resveratrol-treated cells shows that there was a significant difference between the control and resveratrol groups ($P<0.05$). The ROS production level in the resveratrol group was lower than cisplatin although, the difference was insignificant statistically. There was no significant difference between the effects of the two medicines on the ROS production level.

Using Tukey post hoc test, the production of ROS in prostate cancer cells treated with IC50 concentration of cis-platinum and resveratrol was compared with the control group. The mean ROS production in cells treated with cisplatin was 70.90 and resveratrol was 57.89 after three replications and also in the control group was39.87. Tukey post hoc test showed that there was a significant difference between the effects of both drugs with the control group ($P<0.05$), but no significant difference was observed between the two used compounds in the ROS level.

Discussion

In this study, the effects of resveratrol in comparison with cisplatin in various concentrations were examined on the viability of prostate cancer LNCap cell line using the MTT method. Besides, the ROS production level with IC_{50} concentrations of resveratrol and cisplatin on LNCap was assessed using flow cytometry. The results showed that resveratrol can cause cytotoxicity in LNCap cells, and can reduce less ROS compared to cisplatin. This can reveal that both medicines have cytotoxic and anti-cancer properties; although, the property is high in cisplatin as a chemical.

The probability of suffering from prostate cancer for men during their lifetime is 1 in 6 and 1 of 32 people die because of the disease.[28] The disease is highly dependent on lifestyle, the eating style, and the job in addition to genetic factors just similar to other types of cancer. Prostate cancer is heterogenic and varies in terms of grading, genetics, number of chromosomes in the oncogene nucleus, and expression of tumor inhibitory genes. This type of cancer has complicated biological, harmonic, and molecular properties. All of the properties can make some problems with the effective treatment of this cancer.[29] Cisplatin is a medicine, which is widely used for chemotherapy and effective treatment of various types of cancer.[30] However, cisplatin causes cell resistance and severe side effects in normal tissues.[31,32] The side effects include renal toxicity, neurotoxicity, and auditory toxicity. More than 70%

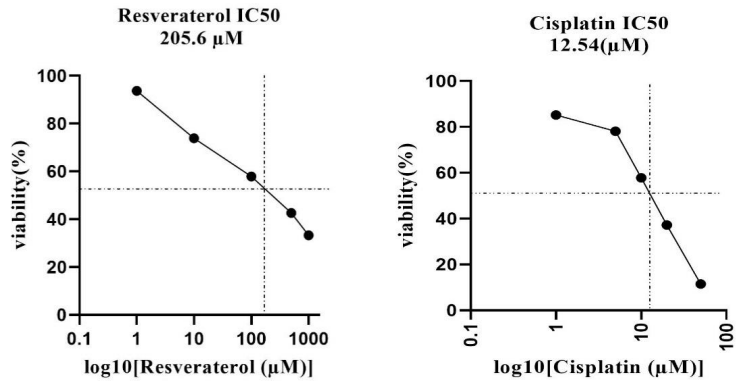

Figure 3. The Diagrams of Determining IC50 Concentration for Cisplatin (Right) and Resveratrol (Left)

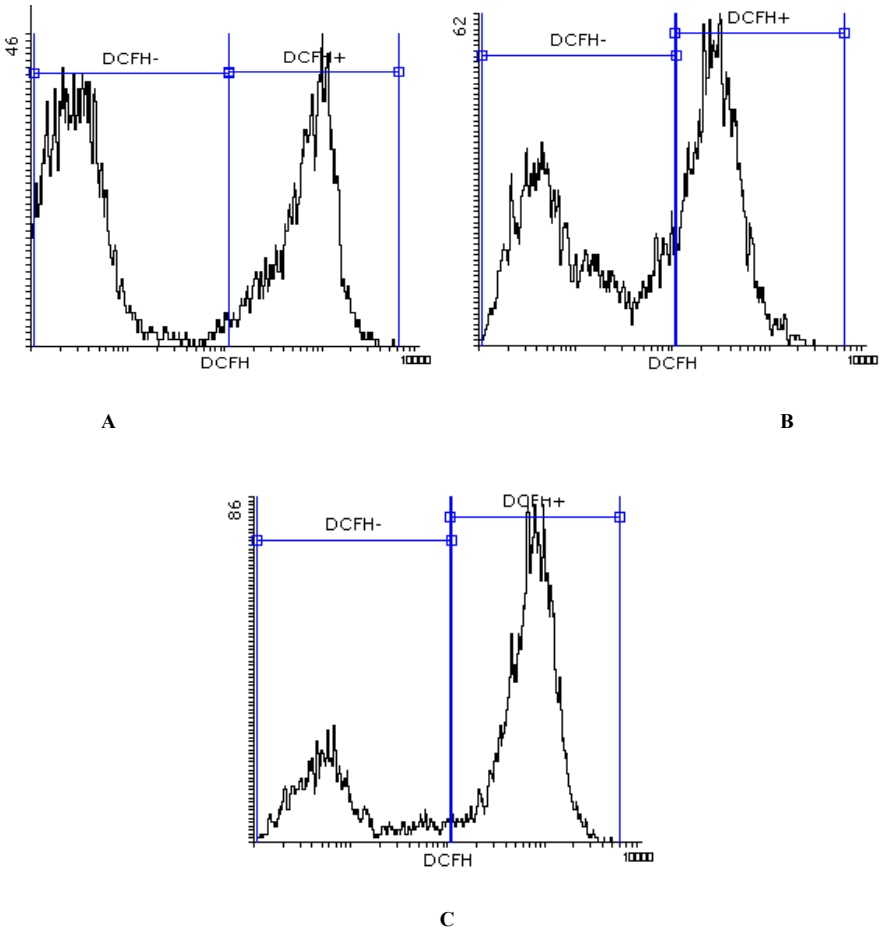

Figure 4. The Histograms Obtained From Flow Cytometry in Control (A), Resveratrol (B), and Cisplatin (C) groups; the length axis (counts) includes cells transferred by the needle from microtube to the device, and its width axis is the percentage of DCFH

of young patients getting cisplatin experience kidney dysfunction.[33] One of the effects of cytotoxicity caused by cisplatin reabsorption is an increase in ROS production, which can activate the internal and external apoptotic pathways, DNA destruction, and lipid peroxidation.[34,35] Many shreds of evidence show that cancer cells produce more ROS, and are exposed to oxidative stress compared to normal cells.[36] Because of the side effects mentioned for cisplatin, scholars are trying to find compounds with the least side effects and most anticancer effects. At present, several compounds such as flavonoids, phenolic acids, terpenes and alkaloids are widely used to treat and prevent diseases such as cancer.[37] Many studies have reported the benefits of phenolic compounds, such as anti-aging, anti-inflammatory, antioxidant and anti-proliferative agents. In addition, the antioxidant enzymes are present to control the oxidants. Products rich in plant polyphenols and polyphenols alter carbohydrate and

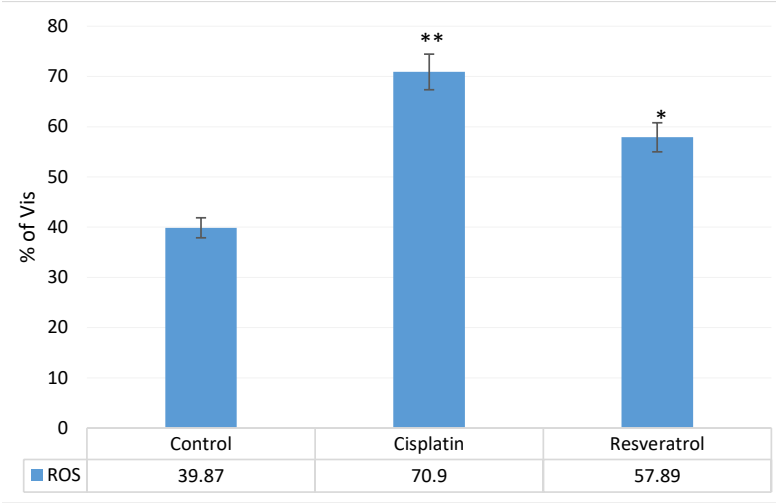

Figure 5. The diagram of ROS induced into the LNCap cells by cisplatin and resveratrol compared to control group (* Significant difference with the control group at the level of $P<0.05$; ** Significant difference with the control group at the level of $P<0.01$)

lipid metabolism, reduce hyperglycemia, dyslipidemia, insulin resistance, improve lipid metabolism, oxidative stress, stress-sensitive signaling pathways, Regulates the inflammatory process.[38] Herbal compounds are underlying candidates in this field. This study has evaluated the effects of resveratrol on the LNCap cancer cell line.

Carter et al studied the effect of resveratrol on cancer treatment. They showed that resveratrol is a natural polyphenol, which can provide some health advantages including metabolism, cardiac protection, and cancer prevention.[39] Ko et al studied the effect of resveratrol on cancer treatment. The study tended to investigate the effect of resveratrol in vivo and in vitro on types of cancer (prostate, breast, lung, colon, skin, and liver). They showed that resveratrol with antioxidant property acts as an anticancer factor, and it is capable to prevent carcinogenicity by inhibiting oxidative stress, inflammation, cancer cell proliferation, and activation of cell death mechanism.[40] Besides, Han et al studied the effects of resveratrol in the treatment of cancers. They showed that resveratrol could enhance the inhibitory effect of the tumor by adjusting the signaling pathways of cell members such as fibroblasts, macrophages, and T cells. Also, it can suppress the malignant phenotypes of cancers created in response to hypoxia, oxidative stress, and inflammation.[41] Madrigal-Perez and Ramos-Gomez conducted a study based on the inhibitory effect of resveratrol on cell respiration. Initial investigations showed the toxic properties of resveratrol against pathogenic plant fungi. However, cell respiration inhibition is a hypothesis, which can explain the toxic and antioxidant properties of resveratrol, because it reduces energy production of pathogenic plant fungi, which can prevent their proliferation. The medicine increases the adenosine monophosphate ratio to adenosine diphosphate. Also, by inhibiting the electron transmission, it can increase ROS production. As a mechanism to overcome oxidative stress, ROS causes antioxidant enzyme expression. In this study, some evidence confirms that cell respiration is the main goal of resveratrol.[42] Nessa et al studied the effect of the combination of resveratrol and cisplatin on ovarian cancer. The results showed that a combination of resveratrol with cisplatin and oxaliplatin can overcome the drug resistance of human ovarian cancer cells.[43]

ROS are a group of highly reactive ions and molecules, which play role in the regulation of types of biologic processes as powerful signaling molecules. Increased ROS production level in cancer cells can be a particular opportunity to destroy the cell. As a result, activating various cell death pathways caused by ROS, or inhibition of cancer cell resistance to chemotherapy using factors increasing ROS production or preventing antioxidant defense can be a good solution for cell death progression in cancer cells.[44]

In a recent review article presenting the beneficial effects of resveratrol in management of insulin resistance we indicated and discussed the mechanism of resveratrol on reduction of ROS production and lipid peroxidation.[45]

The present study showed that resveratrol as an herbal derivative can reduce the viability of cancer cells by creating a cytotoxic property. Also, by increasing ROS production, resveratrol can cause progress of cancer cell death. For this research, in the method section, laboratory kits have been used, all of which had a high price due to the embargo and import of the product.

Conclusion

The results obtained from this study on prostate cancer LNCap cell line, consistent with other experimental studies, showed that resveratrol as a plant antioxidant is capable to cause toxicity in cancer cells and cause the death of these cells. Compared to cisplatin, the cytotoxic effect of resveratrol is considerable and can show its capability

to be used as an anticancer compound. Resveratrol is a plant antioxidant and can produce less ROS compared to cisplatin or control groups. Besides, resveratrol could increase ROS production as an anticancer and pro-apoptosis material compared to the control group. According to the studies in the field of the increase in ROS level to destroy the cancer cells, resveratrol can be used as a plant derivative with controlled concentration to enhance ROS concentration and to create Apoptosis, and reduce the viability of cancer cells. Using the antioxidant property, resveratrol can be used in addition to chemotherapy by cisplatin. Also, resveratrol can be used to prevent drug resistance caused by cisplatin and to decrease non-target cell damage.

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Authors' Contribution

MTG designed and conducted the study. HV and NKJ prepared the proposal and preformed the literature review. NKJ performed the experiment and collected the data. MTG and HV analyzed the data. NKJ and HV prepared the manuscript. MTG supervised the study and revised the manuscript. All authors approved the last version of the manuscript.

Competing Interest

The authors declare that they have no conflict of interest regarding to publication of this article.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

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