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Investigating the Effect of Resveratrol on The Survival Rate of 4T1 Breast Cancer Cells By MTT Method

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ABSTRACT

Cancer is known as an incurable, deadly and deadly disease with pain and disability. During recent decades, changes in people's lifestyles have increased the incidence and prevalence of breast cancer worldwide. With the wide spread of this disease, research has also increased on this issue. Resveratrol is known as a natural polyphenol found in red grapes and some other foods, and with its important anti-cancer properties, it can be effective in reducing the risk of breast cancer and its treatment. Research has shown that resveratrol can have positive effects on breast cancer by reducing DNA methylation, reducing cell proliferation, and reducing cancer growth factors. In this research, resveratrol has been used against 4T1 cancer cells, which were treated with different concentrations of resveratrol after cultivation in 96-well plates and measured by the MTT method. The obtained results showed that resveratrol in all concentrations and at different times significantly reduces the survival of 4T1 cancer cells. By increasing the concentration and treatment time, the decrease in cell viability increases. This indicates strong, dose- and time-dependent effects of resveratrol on breast cancer cells.

1. Introduction

Breast cancer is one of the most common types of cancer in women, and its continuous growth is alarming worldwide. This disease is not only known as a serious threat to women's health but also imposes a great economic and psychological burden on societies. Breast cancer remains one of the most common cancers in women in the world. According to the reports of the World Health Organization, the number of new cases of breast cancer increases annually. In 2020, more than 2.3 million new cases of breast cancer were diagnosed, making it the most common cancer in women. This continuous growth is not only due to the increase in population and the ageing of the population but also due to the improvement of diagnosis methods [1].

Breast cancer consists of different types of cancer cells, each of which has different characteristics and behaviours. Some of the most important breast cancer cell lines include MCF-7: an estrogen-sensitive cell line derived from a 69-year-old woman with breast cancer. These cells are used to study the effects of hormones and hormone treatments. Hormone-resistant cell line MDA-MB-231 derived from a 51-year-old woman with triple-negative breast cancer. These cells are used to study chemical and molecular treatments. 4T1 mouse cell line, which has similar characteristics to human breast cancer and is widely used in animal models to study breast cancer.[2]

The 4T1 cell line is derived from a mouse breast cancer tumour and is used as a suitable model to study the advanced stages of breast cancer due to its ability to metastasize to various organs including the lung, liver and brain. These cells grow rapidly in mice, allowing researchers to study the effects of various drugs and treatments in vitro and in vivo.

Although various methods such as surgery, chemotherapy, radiation therapy and hormonal treatments have been suggested to control the growth and increase of cancer, for the reasons mentioned, these methods are still insufficient and they are facing many challenges such as drug resistance, side effects and metastasis. Over time, many patients develop resistance to chemotherapy and hormonal drugs, which reduces the effectiveness of treatments [3]. Current treatments are often associated with severe side effects that can severely affect patients' quality of life [4]. Many existing treatments are unable to prevent cancer cells from spreading to other organs, which increases mortality.[5]

Resveratrol

Resveratrol is known as a natural polyphenol found in red grapes and some other foods, and with its important anti-cancer properties, it can be effective in reducing the risk of breast cancer and its treatment. Research has shown that resveratrol can have positive effects on breast cancer by reducing DNA methylation, reducing cell proliferation, and reducing cancer growth factors. Also, resveratrol can have protective effects against radiation therapy toxicity and can be used as a useful therapeutic supplement. Dietary intake of resveratrol is associated with a reduced risk of breast cancer. This study was conducted using data from a case-control study in Switzerland and showed that higher consumption of resveratrol was associated with a 50% reduction in the risk of breast cancer[6].

In another study, it was shown that resveratrol can reduce promoter methylation of genes associated with breast cancer. This study, which was conducted on women with a high risk of breast cancer, showed that resveratrol consumption led to a decrease in the methylation of the RASSF-1α gene, which was associated with a decrease in prostaglandin (PGE2) [7]. Resveratrol has antiproliferative effects on breast cancer cells. In laboratory studies, resveratrol has decreased the expression of genes related to DNA methylation and cell proliferation. These effects have been observed in the laboratory environment by reducing the proliferation of breast cancer cells [8]. It also reduces levels of insulin-like growth factor-1 (IGF-I) and IGF-binding protein-3 (IGFBP-3), both of which are associated with cancer growth. A study on healthy volunteers showed that consumption of resveratrol decreased the levels of IGF-I and IGFBP-3 [9]. In a study conducted on the protective effects of resveratrol against skin toxicity caused by radiation therapy in patients with breast cancer, it was shown that taking supplements containing resveratrol reduces skin toxicity in patients undergoing radiation therapy.[10]

Resveratrol, a natural polyphenol found in red grapes and some other foods, has attracted the attention of

many researchers due to its anti-cancer properties. Numerous studies have investigated the effects of this compound on different types of cancer. One of these studies was to investigate the effect of micronized resveratrol (SRT501) in patients with liver metastases from colon cancer. In this study, patients took 5 grams of micronized resveratrol daily for 14 days, and this research aimed to evaluate the safety, pharmacokinetics and pharmacodynamics of this compound. The results showed that micronized resveratrol was well tolerated and higher plasma levels of resveratrol were observed compared to non-micronized resveratrol. Also, a significant increase in the level of activated caspase-3 was reported in malignant liver tissues, indicating the induction of apoptosis by resveratrol [11].

Another study examined the association between dietary resveratrol intake and breast cancer risk. In this case-control study, the relationship between dietary resveratrol consumption and the risk of breast cancer was investigated in 369 cases with breast cancer and 602 controls. The results showed that more consumption of resveratrol was associated with a reduced risk of breast cancer. The odds ratio for the highest level of resveratrol intake was 0.39, indicating a significant reduction in breast cancer risk with resveratrol intake [6].

In another study, the effects of different doses of resveratrol on healthy volunteers were investigated for 29 days. In this study, 40 healthy volunteers took different doses of resveratrol (0.5, 1.0, 2.5, or 5.0 grams daily) for 29 days. This study aimed to evaluate the safety, pharmacokinetics and effect of resveratrol on IGF-I and IGFBP-3 levels. The results showed that resveratrol was well tolerated, but doses of 2.5 and 5 grams caused mild to moderate gastrointestinal symptoms. Consumption of resveratrol caused a significant decrease in IGF-I and IGFBP-3 levels in all volunteers, which indicates the anticancer potential of this compound.[9]

Other researchers have investigated the effects of resveratrol on the methylation of cancer-related genes. In one study, 39 women at high risk of breast cancer were randomized to resveratrol (5 or 50 mg twice daily) or placebo for 12 weeks. The results showed that resveratrol decreased the methylation of the RASSF-1α gene and decreased the expression of prostaglandin (PGE2). These results indicate the anticancer effect of resveratrol through epigenetic changes [7].

Another study investigated the modulation of drug-metabolizing and carcinogenic enzymes by resveratrol. In this study, 42 healthy volunteers consumed 1 gram of resveratrol daily for 4 weeks, and the activity of enzymes in the first and second phases of drug metabolism was evaluated. The results showed that resveratrol decreased the activity of CYP3A4, CYP2D6, and CYP2C9 enzymes, but did not significantly affect the activity of GST and UGT1A1. These results indicate the potential of resveratrol in modulating enzyme systems related to the activation and detoxification of carcinogens [12].

In another study that investigated the clinical pharmacology of resveratrol and its metabolites in patients with colorectal cancer, 20 patients with colorectal cancer were prescribed 0.5 or 1.0 grams of resveratrol daily for 8 days. The levels of resveratrol and its metabolites in human colorectal tissues were measured using HPLC/UV.

The results showed that resveratrol and its metabolites were recovered in colorectal tissues and the use of resveratrol reduced the proliferation of tumor cells. These results indicate the potential of resveratrol as a colorectal cancer prevention agent.[13]

materials and methods

Cell culture:

4T1 cell types were used in this study. These cell types were purchased from the Pasteur Institute of Iran in the form of vials. Mouse tumour cell line 4T1 is one of the few breast cancer cell lines that can metastasize effectively. RPMI-1640 + 10% FBS culture medium was used for the cultivation of these cell lines, which is in the form of powder and contains glutamine and lacks sodium bicarbonate. RPMI-1640 culture medium was purchased from Sigma company.

Examining the cytotoxic property with the MTT method

MTT assay, which stands for MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide), is a colourimetric method based on the reduction and breaking of yellow tetrazolium crystals by succinate dehydrogenase enzyme and the formation of crystals. Purple colour is insoluble.

MTT is soluble in water, which is regenerated by the mitochondria of living cells and turns into a formazan

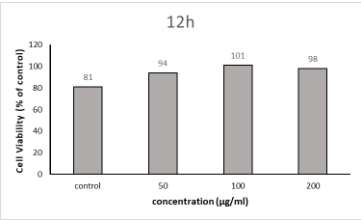
salt insoluble in water. This test is a colourimetric method that examines the activity of cellular enzymes that convert yellow tetrazolium into purple formazan. This test is used to check the rate of cell proliferation and the cytotoxic effects of drugs. In this test, 4T1 cells were first cultivated in small flasks. After the cells reached the expected abundance, the cells were separated from the bottom of the flask. Then, the number of cells was counted using a hemocytometer slide. Then, 200 microliters of cell suspension containing 15 x 103 cells was spread in each well of 96-well plates. In the next step, the cells were treated in triplicate using resveratrol with concentrations of 50, 100 and 200 (g/ml) for 12, 24 and 48 hours. After the incubation time, the entire medium inside the wells was removed, then 100 microliters of complete culture medium and 50 microliters of MTT solution (Roch Diagnostics GmbH, Germany) (2mg/ml) were added to each well. Then the cells were placed in an incubator at 37 degrees and 5% CO2 for 4 hours. After incubation, the medium inside the wells was removed and 200 microliters of DMSO and 25 microliters of Sorenson's buffer were added to each well. Then the plates were incubated for 30 minutes at 37 degrees Celsius in a dark place. Finally, the absorption of the plates was read by the Elizarider device at a wavelength of 570 nanometers. The optical absorbance (OD) recorded from cells containing cells that were near the extract was compared with the optical absorbance of cells that were not under the influence of resveratrol (control cells). The IC50 level (50 per cent inhibition of cell growth) was calculated according to the following formula:

$$\text{IC50 (\%)} = \frac{\text{OD sample} - \text{OD control cells}}{\text{OD control cells}} \times 100$$

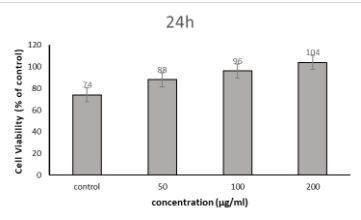
Determining the viability of cells by trypan blue staining:
Measuring cell viability and growth by the trypan blue method is an easy method to evaluate cell membrane integrity and as a result their proliferation or death. For this test, 5 x 104 cells were distributed in each well of a 24-well plate, and after treating the cells with resveratrol, the cells were counted using trypan blue dye. The percentage of living cells was calculated with the following formula:

$$\text{The percentage of living cells (\%)} = \frac{\text{Average number of live cells}}{\text{Average number of dead cells}} \times 100$$

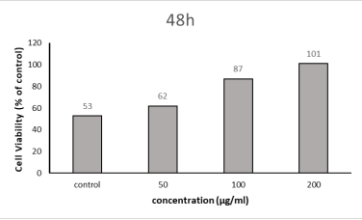
Results



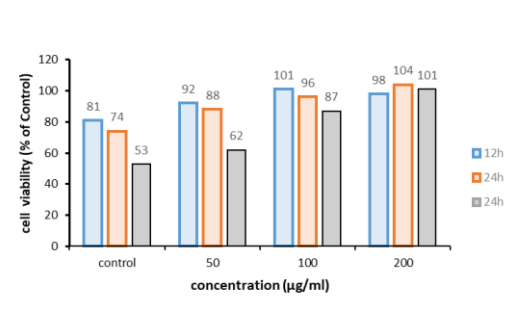
(A)



(B)



(C)



(D)

Cytotoxic effect of resveratrol on 4T1 cells
A) It shows the effect of resveratrol in 12 hours. B) It shows the effect of resveratrol in 24 hours.
C) It shows the effect of resveratrol in 48 hours.
D) It shows the comparison of the decrease in viability of 4T1 cells at 12, 24 and 48 hours.

The results show that resveratrol at a concentration of 50 μg/ml/12 hours decreased cell viability, but this decrease was not significant. But with increasing concentration, its effect increased. During 24 hours of treatment, the survival percentage of cancer cells was significantly affected by increasing the concentration, and at this time IC50 was equal to 1.15 ± 4.685 μg/ml. Also, after 48 hours of treatment, the effect of resveratrol reached its maximum and IC50 at this time was equal to 6.752 ± 1.01 μg/ml.

.Table (1): shows the IC50 values of resveratrol on 4T1 cells at 12, 24 and 48 hours

	IC50 (μg/ml)		
	12h	24h	48h
4T1	1.54 ±3.465	1.15 ±4.685	1.01 ±6.752

The results of this research showed that resveratrol with different concentrations (50, 100, and 200 μg/ml) at different times (12, 24, and 48 hours) decreases the viability of 4T1 cancer cells. IC50 values showed that the cytotoxic effects of resveratrol increased in longer times and with increasing concentration. At 12 hours, IC50 was equal to 3.465 ± 1.54 μg/ml, which increased to 4.685 ± 1.15 and 6.752 ± 1.01 μg/ml at 24 and 48 hours, respectively.

These results show that resveratrol can act as an effective cytotoxic agent on 4T1 breast cancer cells and these effects are enhanced with increasing time and concentration. Therefore, resveratrol can be used as a therapeutic supplement to reduce the growth of cancer cells.

Discussion:

The results of this study show that resveratrol, as a natural polyphenol found in red grapes and some other foods, has important anti-cancer properties. The cytotoxic effects of resveratrol on 4T1 cancer cells indicate the potential of this compound in preventing the proliferation and survival of cancer cells.

Various studies also support the anti-cancer properties of resveratrol. For example, research has shown that resveratrol can have positive effects on breast cancer by reducing DNA methylation and reducing cell proliferation [6]. Also, a study has shown that the consumption of resveratrol reduces the methylation of cancer-related genes and this effect is related to the reduction of prostaglandin (PGE2) [7]. In this regard, other studies have investigated the effect of resveratrol on the immune system and the reduction of cancer growth factors. For example, one study found that resveratrol reduced levels of IGF-I and IGFBP-3, both of which are associated with cancer development [9].

The anticancer effects of resveratrol are exerted through different mechanisms. Studies have shown that resveratrol can reduce promoter methylation of cancer-related genes, leading to decreased cell proliferation and increased apoptosis.[14]

Also, resveratrol reduces the expression of genes related to cell proliferation and thus inhibits the proliferation of cancer cells [8]. Resveratrol decreases the levels of IGF-I and IGFBP-3, both of which are associated with cancer development [9].

Consumption of resveratrol can have protective effects against radiation therapy toxicity and can be used as a useful therapeutic supplement [10, 15].

The results of this study, which show that resveratrol has anticancer effects on 4T1 breast cancer cells, are consistent with the findings of many previous studies. A study by [7] showed that resveratrol can reduce promoter methylation of cancer-related genes. These effects lead to decreased cell proliferation and increased apoptosis in cancer cells. The results of this study also showed that resveratrol decreases the survival of 4T1 cancer cells, which is consistent with the findings of this researcher.

A study by [16] showed that the consumption of resveratrol can have protective effects against radiation therapy toxicity. The results of this study also showed that resveratrol can act as an effective complementary treatment agent

In a study by [17], it was shown that resveratrol can reduce cancer cell metastasis and increase apoptosis. This study also showed that resveratrol increases the induction of apoptosis in cancer cells by increasing its concentration. A study by [13, 18] showed that resveratrol reduces the proliferation of colorectal cancer cells. These results show the broad effect of resveratrol on different types of cancers, and the results of this study also confirm these effects.

The summary of the results of this study is consistent with many other researches in the field of anticancer effects of resveratrol. This agreement shows that resveratrol has a high potential to be used as an adjunctive therapy against various cancers, including breast cancer. Further studies are needed to investigate the mechanisms of action and optimize the dosages so that this compound can be fully used in clinical treatments.

Although the results of this study and previous studies indicate the anticancer potential of resveratrol, some limitations should be considered in future research. Most of the studies conducted on cancer cells were in laboratory conditions and there is a need for more clinical research to confirm the effects of resveratrol in the human body. A more detailed investigation of side effects and determination of optimal doses for resveratrol consumption is of great importance.

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