Egyptian Journal of Veterinary Sciences

Resveratrol Inhibits Cell Cycle Dynamics, Caspase Activation, and Programmed Cell Death: Implications for Cancer Treatment in MCF-7 Cells"

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Abstract

Resveratrol is a plant-derived polyphenol known for its anti-inflammatory and anti-tumor properties in laboratory models and living organisms. Recent studies suggest that certain analogs of resveratrol may exhibit more effective anti-tumor factors. This study investigates the impact of resveratrol on the cell cycle of MCF-7 cells at concentrations of 1.5 and 2μg\ ml. Resveratrol, at 2μg\ml, significantly alters the cell cycle distribution, indicating a concentration-dependent effect. Doxorubicin serves as a positive control and induces G1 phase arrest. These results contribute to understanding the potential mechanisms of resveratrol in cancer treatment and shed light on its impact on cell cycle dynamics. Additionally, the study explores the effects of different concentrations of resveratrol on the activity of Caspase 8, using doxorubicin as a positive control for comparison. Human cell lines were exposed to varying concentrations of resveratrol (1.5 μg\ ml and 2 micrograms/ml), and Caspase 8 activities were compared with both untreated cells (control group) and those treated with doxorubicin. The average Caspase 8 activity and standard deviation were calculated for each treatment group, and the statistical significance was determined. this study highlights the concentration-dependent effects of resveratrol on the cell cycle distribution of MCF-7 cells, suggesting its potential as a therapeutic agent in cancer treatment. Furthermore, the investigation of Caspase 8 activity provides additional insight into the anti-cancer properties of resveratrol in breast cells.

Keywords: Resveratrol, Cell cycle dynamics, Caspase activation, MCF-7 cells, Doxorubicin, Apoptosis, Mitochondrial membrane**.**

Introduction

Resveratrol (trans-3, 4′, 5-trihydroxystilbene), originally identified as a naturally occurring anti-tumor molecule, is a phytoalexin polyphenol produced by several plants, including grapes, berries, and others [1, 2]. This polyphenolic compound, found in grape skin and certain other natural products like knotweed, has demonstrated the ability to delay or prevent carcinogenesis stages [3,4]. This preventive effect may be associated with resveratrol's ability to halt cell cycle progression [5-7]. Breast cancer stands as one of the most prevalent malignant tumors globally and ranks second as a cause of cancer-related deaths among women in the United States [8, 9]. The development of breast tumors is primarily influenced by a family history, and various lifestyle factors may also contribute to breast cancer, such as occupational status, obesity, smoking, alcohol consumption, menopausal age, and high blood pressure [10, 11]. or induce programmed cell death in cancer cells through apoptosis [12-14]. Studies have provided insights into the anti-aging health benefits of resveratrol, including improved metabolism, heart protection, and cancer prevention. Pre-clinical studies have revealed the biological effects of resveratrol in various aspects. However, its

broad application in clinical treatment remains challenging [17,16,15]. Resveratrol restricts the survival of breast cancer stem-like cells and induces autophagy [18, 19], a highly conserved physiological cellular process crucial for nuclear organisms, participating in cell cycle regulation and cellular component turnover [20,21]. Unlike autophagy, programmed cell death is characterized by membrane bubbling, DNA fragmentation, and distinct apoptotic bodies. Programmed cell death requires the activation, expression, and regulation of genes; It is not a pathological condition but an adaptive and proactive mechanism in response to environmental changes and cellular rejuvenation, Doxorubicin is commonly used to treat various types of cancer, including bladder, breast, stomach, lung, ovarian, thyroid, and soft tissue cancer. It works by damaging the genes of cancer cells and interfering with their reproduction [24].

Contrary to autophagy, programmed cell death involves mitochondrial pathways to induce the release of soluble particles from the intermembrane space. One of these particles is cytochrome c, which, in the cellular cytoplasm, stimulates the sequestration of a small molecule called Apaf-1 to generate a compound where caspase-9 is activated. Active caspase-9 then stimulates the initiating maturation of caspase-3 and other terminal caspases, leading to cell death. The release of cytochrome c can be prevented by anti-apoptotic Bcl-2 family proteins, which are supposed to maintain the integrity of the mitochondrial membrane. Pro-apoptotic members of the Bcl-2 family, known as BH3-only proteins, act as cellular damage sensors and initiate the process of death either by inhibiting anti-apoptotic family members or activating another set of pro-apoptotic proteins associated with Bcl- 2. The proteins, Bax/Bak, induce conformational changes by provoking cell-compatible alterations [28, 29].

Several reports have shown that resveratrol can stimulate programmed cell death in cancer cells through a mitochondria-dependent pathway partially relying on Bax-associated conformational changes and cellular redistribution [30,31]. This study aims to shed light on the role of naturally isolated resveratrol from grape skins in the cell cycle and its impact on caspase-9 and caspase-8 enzymes.

Material and Methods

Preparation of Resveratrol

This experiment was carried out in the period from March 2022 to June 2023 At the Biotechnology Center in Baghdad, Iraq, a grape peel extract was prepared according to [32], with all steps conducted away from direct light and high pressure to prevent plant extract oxidation. Approximately 500 grams of fresh grapes were macerated with 2.5 liters of 80% ethanol in a cool and dark place for 72 hours. The extract was filtered, and the filter was dried at 30-40 °C

using a rotary evaporator to obtain 1/10 of its original volume, which was stored at -20°C. The method described in [33] was employed for phenol isolation and purification.

Cell Cycle Assay

To study the cell cycle of MCF-7 breast cancer cells, cells were obtained from the Cancer Research Center at the University of Mustansiriya, Iraq. MCF-7 cells, isolated from a 69-year-old Caucasian woman with breast cancer in 1970, were used. MCF-7 is an abbreviation for the Michigan Cancer Foundation-7. The CycleTest™ Plus DNA Reagent kit was used for the cell cycle analysis. Cells were seeded at a concentration of 5 x 105 cells per well using a 12-well plate and complete growth medium. After 24 hours of incubation at 37°C and 5% CO_2, the culture medium was replaced with resveratrol at concentrations of 1.5, 2, and 2.5 µg\ml for 24 hours to analyze the cell cycle. Doxorubicin was used as a positive control at a concentration of 1 µM, following the protocol in [34].

Caspase Assay

The Caspase-Glo® 8 and 9 assays were used to measure the activity of Caspase 8 and Caspase 9, crucial in cell growth, differentiation, and programmed cell death. Cells were cultured in a 96-well plate and incubated at 37°C for 24 hours. After incubation, the culture medium was replaced, and cells were treated with resveratrol at concentrations of 2 and 2.5 µg\ ml for 24 hours. Doxorubicin was used as a positive control at a concentration of 1 µM, and DMSO served as a negative control. The Caspase-Glo® reagent was added, and luminescence was measured at 405 nanometers to assess Caspase activity [35].

Statistical analysis

Statistical analysis was performed using GraphPad Prism software (Version 9, GraphPad Software Inc, La Jolla, CA). Data were analyzed using one-way analysis of variance (ANOVA), and mean comparisons were made using the Tukey multiple comparison test. Significance was considered at *$p < 0.05$ and **$p < 0.01$.

Results and Discussion

Effect of Resveratrol Particles on the Cell Cycle of MCF-7 Cells

The impact of resveratrol on the cell cycle of MCF-7 breast cancer cells was investigated using flow cytometry after treating the cells with various concentrations of resveratrol (1.5 and 2 micrograms/ml) for 24 hours. The results (Table 1) demonstrated no significant effect of the tested concentrations of resveratrol on different phases of the cell cycle in MCF-7 cells compared to the control

model (untreated cells). However, when comparing the results with the positive control model (Doxorubicin drug), the experiment revealed the impact of two substances, resveratrol and doxorubicin, on the cell cycle stages of MCF-7 cells. The cell cycle stages involve the distribution of cells in the G1, S, and G2/M phases, and the percentage of cells in each stage was reported for different treatments in μg\ ml.

Resveratrol at a concentration of 1.5 μg\ ml showed no significant change in the distribution of cells in the G1, S, and G2/M phases compared to untreated cells. Resveratrol at 2 μg\ ml indicated a significant increase in the S phase, suggesting a potential effect on DNA synthesis and cell proliferation. Doxorubicin (1mM) showed a significant increase in the G1 phase, indicating cell cycle arrest, a distinct response to DNA damage induced by doxorubicin. This aligns with its role as a positive control, validating the experimental setup. These results indicate that resveratrol, especially at a concentration of 2 μg/ml, may affect the cell cycle of MCF-7 cells by reorganizing the amino acid sequences in the DNA and returning them to their original pre-tumor nature. On the other hand, doxorubicin, a well-known chemotherapeutic agent, induces cell cycle arrest in the G1 phase, consistent with its mechanism of action. Further studies are warranted to explore specific molecular pathways involved and assess the potential of resveratrol as a modulator of cell cycle progression in cancer cells. The notable increase in the G1 phase with doxorubicin indicates a mechanism of cell cycle arrest, consistent with its known toxic effects on cells. Resveratrol, at the tested concentrations, exhibits a concentration-dependent effect on the cell cycle, with a significant impact observed at 2 μg\ml. The results suggest that resveratrol may influence the cell cycle progression in MCF-7 cells, potentially affecting cell proliferation and survival.

Resveratrol treatment of MCF-7 cells demonstrates a dose-dependent inhibition of cell growth and accumulation in the S phase of the cell cycle at low concentrations. However, higher concentrations do not stimulate S phase accumulation [36]. Resveratrol inhibits the proliferation of MCF-7 cells in a time- and dose-dependent manner, with efficacy unrelated to caspase expression [37]. Researchers have also shown that Resveratrol enhances time-dependent cell death in MCF-7 cells, regardless of caspase-3 expression. Significantly, there is a pronounced cell cycle arrest in the S phase in both cell groups. Resveratrol negatively regulates the growth of prostate cancer cells by impacting the generation process and promoting programmed cell death in the prostate [38,39], and resveratrol prevents cell proliferation and stimulates programmed cell death in human breast cancer cells (MCF-7) [40].

These findings collectively underscore the multifaceted effects of Resveratrol on cancer cells, demonstrating its potential as a therapeutic agent for breast and prostate cancer by inhibiting cell growth, inducing cell cycle arrest, and promoting programmed cell death. Further research is warranted to elucidate the specific molecular pathways involved and explore the translational potential of Resveratrol in the treatment of cancer.

Resveratrol's Impact on Cellular Indicators

Figure 2 illustrates some cellular features investigated to assess the effect of Resveratrol on membrane permeability. A noticeable increase in nuclear density was observed when cells were treated with the compound at concentrations of 1.5 and 2 μg\ml, showing significant differences ($p \geq 0.01$) compared to control cells. No significant impact was observed for other concentrations. Nuclear density levels were presented relative to the control treatment, the fourth indicator studied was the strength of mitochondrial membranes, crucial for their integrity. Membrane depolarization serves as an ideal indicator for damage, increasingly affected by drug toxicity, offering an effective description of cell death signals [5]. Results in Figure2 show that Resveratrol concentrations of 1.5 and 2 μg\ ml led to a significant decrease in mitochondrial membrane strength ($p \geq 0.01$) compared to the control. This suggests no significant difference in mitochondrial membrane strength when treated with Boxo. at concentrations of 1.5 and 2 μg\ml.

Cytochrome C, a major component in the weakly bound electron transport chain on the outer layer of the inner mitochondrial membrane, plays a crucial role in programmed cell death [41]. Results in Figure 2 indicate no statistically significant differences between concentrations of 1.5 μg\ml and 2 μg\ ml compared to the control. However, Cytochrome C levels significantly increased ($p \geq 0.01$) compared to the control at concentrations of 1.5 and 2 micrograms/ml. The results suggest that high concentrations of the compound affect all cellular indicators in MCF-7 cancer cell lines when exposed for 24 hours at 37 °C, inducing cells to undergo programmed cell death (Figure2). Early features of programmed cell death include mitochondrial activity imbalance, changes in membrane permeability, and alterations in the oxidative and reduction system within, leading to pore opening and allowing the passage of ions and small molecules through the membrane. The opening of pores in the cell membrane leads to ionic imbalance, causing the dissociation of the respiratory chain and releasing Cytochrome C into the cell [42].

The use of the cellular toxicity assay for drugs is an essential part of detecting new medications. It is a complex process that affects multiple metabolic

pathways after cells are exposed to a toxic substance, leading to cell death. Cell death can occur either as programmed cell death or necrosis, typically accompanied by changes in nuclear shape, cell permeability, and mitochondrial function, resulting in the loss of mitochondrial membrane function and the release of Cytochrome C from the cell mitochondria [43]. Resveratrol demonstrated cellular toxicity towards MCF-7 cancer cell lines by inducing nuclear fragmentation, chromatin condensation, and entry into programmed cell death. This is a result of increased production of free oxygen radicals after-treatment and the release of damaged mitochondrial debris [45]. Resveratrol prevents cell survival, promotes programmed cell death in MCF-7 cells [46], and enhances the anti-cancer effects of paclitaxel in human liver cancer cells HepG22. Resveratrol induces intrinsic cell death dependent on caspases associated with increased oxidative stress mediated by oxygen and nitrogen in its cells [47]. Ginkgolide B, a compound stimulating programmed cell death in MCF-7 cells, regulates the permeability of the outer mitochondrial membrane and releases mitochondrial Cytochrome C [48]. MitoVitE, an antioxidant targeting mitochondria, reduces oxidative stress caused by paclitaxel and potential changes in the mitochondrial membrane in MCF-7 cells [49].

However, it remains clear how the effects of Resveratrol are related to the post-cellular Francisco system and the specific parameters mentioned in the question. Further research is needed to determine the effects of Resveratrol concentrations on cellular knowledge content, cell membrane permeability, mitochondrial membrane permeability, and the level of Cytochrome C release in MCF-7 linear cancer cells post-treatment.

Effect of Resveratrol on Caspases 8 and 9

Caspase 8 and Caspase 9, enzymes belonging to the cysteinyl aspartate-specific enzyme family, activate during the cell's entry into programmed cell death. Figure 3 shows that MCF-7 cells exhibit a dose-dependent increase in Caspase 8 activity after 24 hours of treatment. Resveratrol at concentrations of 1.5 and 2 µg\ml resulted in a significant increase in Caspase 8 activity by 1.64-fold and 2.13-fold, respectively ($p \leq 0.01$), compared to the control model (untreated cells).

Figure 3 illustrates the impact of Resveratrol on Caspase 8, with the control model showing an average Caspase 8 activity of $53,882 \pm 3,328$ units, serving as a baseline reference. At a concentration of 1.5 micrograms/ml, Resveratrol significantly increased Caspase 8 activity, reaching an average of $73,002 \pm 3,177$ units (<0.0001, **). Further escalation of Resveratrol concentration to 2 µg\ml led to a more pronounced elevation in Caspase 8 activity, with an average of $73,002 \pm 3,177$ units

(<0.0001, **). The positive control model, Doxorubicin, exhibited the highest Caspase 8 activity among all tested conditions, with an average of $86,994 \pm 5,810$ units (<0.0001, **). This robust response indicates Doxorubicin's strong proapoptotic effect on the studied cell lines (Figure 4). The significant dose-dependent increase in Caspase 8 activity in response to Resveratrol suggests its potential role as a stimulator of programmed cell death in the studied cell lines. The statistical differences compared to the untreated control model support the idea that Resveratrol, even at relatively low concentrations, has a measurable effect on Caspase 8 activation, a crucial mediator of programmed cell death pathways.

Figure 4 demonstrates the impact of Resveratrol on Caspase 9 activity. The control model displayed a baseline Caspase 9 activity of 15,833 units. Treatment with Resveratrol at a concentration of 1.5 µg\ml resulted in an increased Caspase 9 activity, with an average of $22,198 \pm 4,746$ units ($p = 0.0213$, *). Further escalation of Resveratrol concentration to 2 µg\ ml led to a more pronounced enhancement of Caspase 9 activity, reaching an average of $34,447 \pm 3,068$ units ($p = 0.0008$, **). The positive control model treated with Doxorubicin exhibited a significant increase in Caspase 9 activity, recording an average of $67,559 \pm 2,629$ units ($P < 0.0001$, **). The notable increase in Caspase 9 activity in response to Resveratrol suggests its potential role in stimulating programmed cell death through the activation of the intrinsic apoptotic pathway. The statistical significance of differences compared to the untreated control model supports the idea that Resveratrol, even at relatively low concentrations, has a measurable effect on Caspase 9 activation, a key initiator of the intrinsic apoptotic cell death pathway. The comparison with the positive control model, Doxorubicin, provides context for the effectiveness of Resveratrol in stimulating Caspase 9 activity. While Resveratrol shows a significant effect, the positive control model demonstrates higher and more potent activation of Caspase 9, indicating that Doxorubicin has a stronger impact on the intrinsic apoptotic cell death pathway in the studied cell lines

Resveratrol demonstrates a dose-dependent inhibition of Caspase activation and enhances cell viability with a 50% inhibitory concentration (IC50) of 66.3 ± 13.81 micrometers in mouse primary fibroblasts [50]. Resveratrol promotes caspase-3 and PARP cleavage, proteins associated with programmed cell death, and reduces dose-dependent protein levels [51]. Resveratrol-induced programmed cell death is evident through G1 cell cycle arrest, increased Bax release, and activation of Caspase 3 in MCF-7 cells [52]. Resveratrol stimulates caspase-dependent programmed cell death through increased

oxidative stress via oxygen and nitrogen in MCF-7 cells [53,54]. Resveratrol induces autophagy-dependent programmed cell death in HL-60 cells through intrinsic and extrinsic programmed cell death pathways. Resveratrol modulates mitochondrial membrane potential and markers associated with programmed cell death, such as increased Bax/Bcl-2 ratio and cleaved forms of Caspase 8 and Caspase 9 [55]. Resveratrol induces programmed cell death by modifying the crosstalk between p53 and Sirt-1 in the tumor microenvironment of CRC [56-58].

Conclusions

The study provides valuable insights into the impact of Resveratrol and Doxorubicin on cell cycle dynamics in MCF-7 cells, contributing to our understanding of their potential mechanisms of action in cancer treatment. Further investigations are justified to elucidate the underlying molecular pathways behind these effects and explore the therapeutic effects of Resveratrol in cancer treatment. This study highlights the concentration-dependent impact of Resveratrol on Caspase 8 activity, shedding light on its ability to induce programmed cell death in human cell lines. The results emphasize the need for continued research to uncover the fundamental molecular mechanisms and assess the translational effects of Resveratrol in cancer treatment and other contexts where the regulation of programmed cell death is crucial. The findings shed light on the need for further exploration of Resveratrol's mechanisms and potential synergies with other programmed cell death regulators. This knowledge contributes to a better understanding of the therapeutic potential of Resveratrol, especially in contexts where internal programmed cell death is a crucial regulatory process, as seen in cancer treatment.
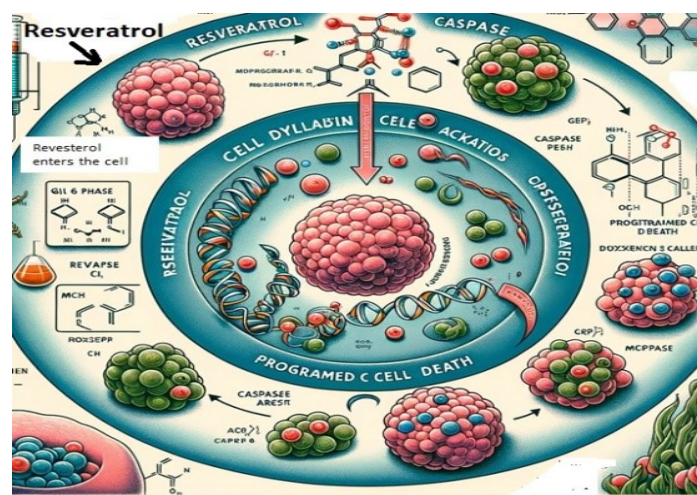

Fig. 1. Schematic presentation of the effect of Revesterol on the cell life cycle and caspase 8,9 enzymes

TABLE 1. Effect of Resveratrol and Doxorubicin as a Positive Control Model on the Cell Cycle of MCF-7 Cell. (mean±SD)

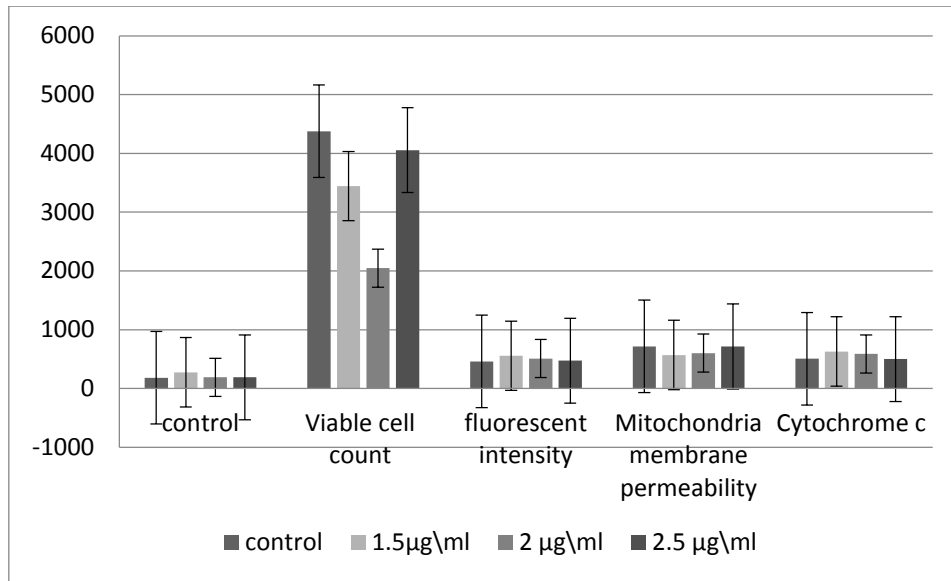| Treatments	Cell Cycle phases (Phases distribution Cell count%)		
(µg mL^{-1})	G$_1$	S	G$_2$/M
Untreated Cells	55.85 ± 0.35	19.55 ± 2.75	18.85 ± 0.91
1.5 (µg mL^{-1})	57.55 ± 0.45 NS	22.75 ± 3.46 NS	18.95 ± 2.05 NS
2 (µg mL^{-1})	52.7 ± 0.65 N	26.25 ± 1.90 N	25.65 ± 1.48 NS
Doxorubicin 1 mM	62.15 ± 1.48 **	21.55 ± 2.05 NS	18.5 ± 0.7 NS

Fig. 2. Resveratrol's Impact on Cellular Knowledge Content, Cellular Membrane Permeability, Mitochondrial Membrane Permeability, and Cytochrome C Release Levels in MCF-7 Cancer Cells Incubated for 24 hours at 37°C.
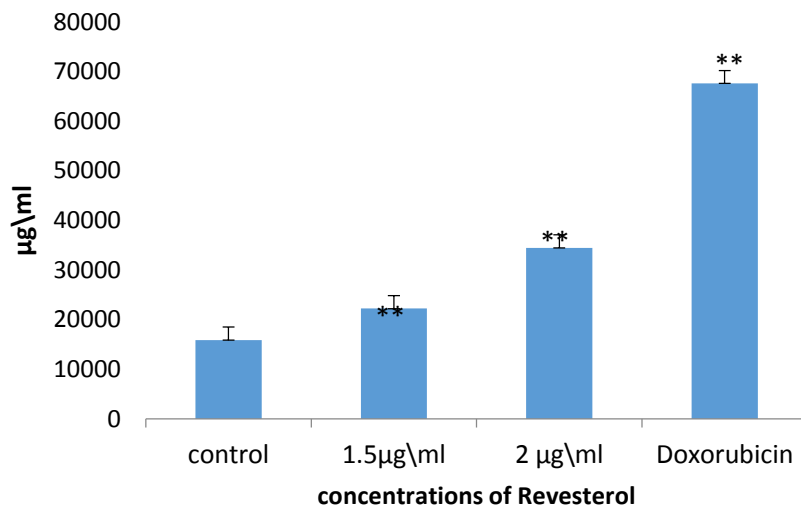

Fig. 3. The effect of different concentrations of Revesterol and the positive control model on Caspase 8 activity compared to the control model cells without treatment (mean±SD).

Fig. 4. The effect of different concentrations of Revesterol and the positive control model on Caspase 9 activity compared to the control model cells without treatment (mean±SD).
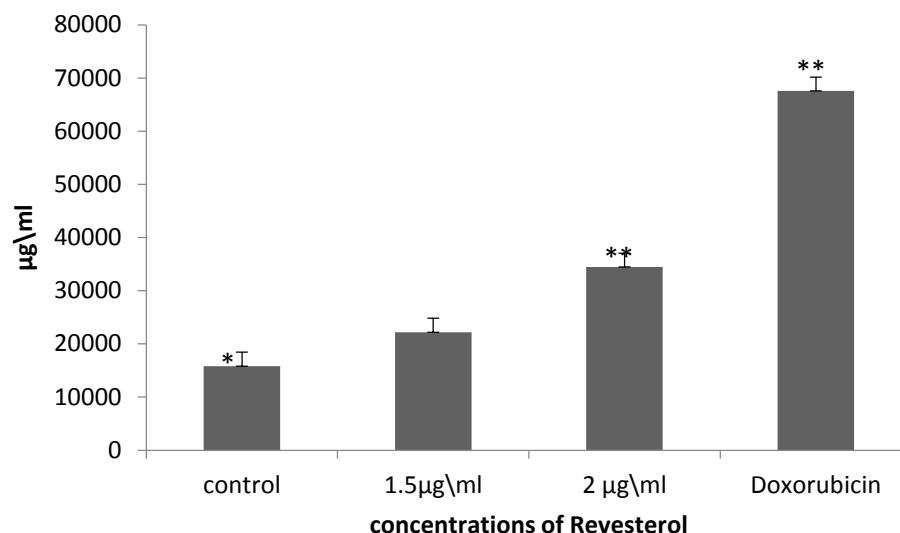
Conflict of interest

None

Authors contributions: Sura H. Hamad and Zinah Hashim Mosleh: Practical work, Kawther M. Nasir: statistical analysis and Ashwaq T. Hameed: Manuscript writing and editing

References

1. Liang, Y. C., Tsai, S. H., Chen, L., Lin-Shiau, S. Y. and Lin, J. K. Resveratrol-induced G2 arrest through the inhibition of CDK7 and p34CDC2 kinases in colon carcinoma HT29 cells. *Biochemical Pharmacology*, **65**(7), 1053-1060(2003). https://doi.org/10.1016/S0006-2952(03)00011-X

2. Schneider, Y., Duranton, B., Goss, F., Schleiffer, R., Seiler, N. and Raul, F. Resveratrol inhibits intestinal tumorigenesis and modulates host-defense-related gene expression in an animal model of human familial adenomatous polyposis. *Nutrition and Cancer*, **39**(1), 102-107(2001). https://doi.org/10.1207/S15327914nc391_14

3. Sharifi-Rad, J., Quispe, C., Durazzo, A., Lucarini, M., Souto, E. B., Santini, A. and Cruz-Martins, N. Resveratrol' biotechnological applications: Enlightening its antimicrobial and antioxidant properties. *Journal of Herbal Medicine*, **32**, 100550 (2022). https://doi.org/10.1016/j.hermed.2022.100550

4. Chen, H. Y., Lin, P. H., Shih, Y. H., Wang, K. L., Hong, Y. H., Shieh, T. M. and Hsia, S. M. Natural antioxidant resveratrol suppresses uterine fibroid cell growth and extracellular matrix formation in vitro and in vivo. *Antioxidants,* **8**(4), 99(2019). https://doi.org/10.3390/antiox8040099

5. Mbemi, A., Khanna, S., Njiki, S., Yedjou, C. G. and Tchounwou, P. B. Impact of gene–environment interactions on cancer development. *International Journal of Environmental Research and Public Health*, **17**(21), 8089(2020).

6. Daly, A. A., Rolph, R., Cutress, R. I. and Copson, E. R. A review of modifiable risk factors in young women for the prevention of breast cancer. *Breast Cancer: Targets and Therapy,* 241-257(2021). https://doi.org/10.2147/BCTT.S268401

7. Esmaeilzadeh, A. A. and Nasirzadeh, F. Investigation of Chemicals on Breast Cancer. *Eurasian Journal of Chemical, Medicinal and Petroleum Research,* **1**(5), 51-75(2022). https://doi.org/10.5281/zenodo.8020

8. Tekos, F., Makri, S., Skaperda, Z. V., Patouna, A., Terizi, K., Kyriazis, I. D. and Demetrios, K. Assessment of antioxidant and antimutagenic properties of red and white wine extracts in vitro. *Metabolites,* **11**(7), 436(2021). https://doi.org/10.3390/metabo11070436

9. Grinan-Ferre, C., Bellver-Sanchis, A., Izquierdo, V., Corpas, R., Roig-Soriano, J., Chillón, M. and Pallas, M. The pleiotropic neuroprotective effects of resveratrol in cognitive decline and Alzheimer's disease pathology: From antioxidant to epigenetic therapy. *Ageing Research Reviews*, **67**, 101271(2021). https://doi.org/10.1016/j.arr.2021.101271

10. Rytsyk, O., Soroka, Y., Shepet, I., Vivchar, Z., Andriichuk, I., Lykhatskyi, P. and Lisnychuk, N. Experimental evaluation of the effectiveness of resveratrol as an antioxidant in colon cancer prevention. *Natural product Communications,* **15**(6), 1934578X20932742(2020). https://doi.org/10.1177/1934578X20932742

11. Gianchecchi, E. and Fierabracci, A. Insights on the Effects of Resveratrol and Some of Its Derivatives in Cancer and Autoimmunity: A Molecule with a Dual Activity. *Antioxidants (Basel, Switzerland),* **9**(2), 91(2020). https://doi.org/10.3390/antiox9020091

12. Kulashekar, M., Stom, S. M. and Peuler, J. D. Resveratrol's Potential in the Adjunctive Management of Cardiovascular Disease, Obesity, Diabetes, Alzheimer Disease, and Cancer. *The Journal of the American Osteopathic Association,* **118**(9), 596–605(2018). https://doi.org/10.7556/jaoa.2018.133

13. Wei, L., Chai, S., Yue, C., Zhang, H., Li, J. and Qin, N. Resveratrol protects osteocytes against oxidative stress in ovariectomized rats through AMPK/JNK1-dependent pathway leading to promotion of autophagy and inhibition of apoptosis. *Cell Death Discovery*, **9**(1), 16(2023).

14. Solary, E., Bettaieb, A., Dubrez-Daloz, L. and Corcos, L. Mitochondria as a target for inducing death of malignant hematopoietic cells. *Leukemia & lymphoma*, **44**(4),563-574(2003). https://doi.org/10.1080/1042819021000038001

15. Meng, X., Zhou, J., Zhao, C. N., Gan, R. Y. and Li, H. B. Health Benefits and Molecular Mechanisms of Resveratrol: A Narrative Review. *Foods (Basel, Switzerland),* **9**(3), 340(2020). https://doi.org/10.3390/foods9030340

16. Salehi, B., Mishra, A. P., Nigam, M., Sener, B., Kilic, M., Sharifi-Rad, M., Fokou, P. V. T., Martins, N. and Sharifi-Rad, J. Resveratrol: A Double-Edged Sword in Health Benefits. *Biomedicines,* **6**(3), 91(2018). https://doi.org/10.3390/biomedicines6030091

17. Fu, Y., Chang, H., Peng, X., Bai, Q., Yi, L., Zhou, Y., Zhu, J. and Mi, M. Resveratrol inhibits breast cancer stem-like cells and induces autophagy via suppressing Wnt/β-catenin signaling pathway. *PloS one,* **9**(7), e102535(2014). https://doi.org/10.1371/journal.pone.0102535

18. Bhaskara, V. K., Mittal, B., Mysorekar, V. V., Amaresh, N. and Simal-Gandara, J. Resveratrol, cancer and cancer stem cells: A review on past to future. *Current Research in Food Science,* **3**, 284-295(2020). https://doi.org/10.1016/j.crfs.2020.10.004

19. El-Readi, M. Z., Eid, S., Abdelghany, A. A., Al-Amoudi, H. S., Efferth, T. and Wink, M. Resveratrol mediated cancer cell apoptosis, and modulation of multidrug resistance proteins and metabolic enzymes. *Phytomedicine,* **55**, 269-281 (2019). https://doi.org/10.1016/j.phymed.2018.06.046

20. Haque, A., Brazeau, D. and Amin, A. R. Perspectives on natural compounds in chemoprevention and treatment of cancer: an update with new promising compounds. *European Journal of Cancer,* **149**, 165-183(2021). https://doi.org/10.1016/j.ejca.2021.03.009

21. Dalpiaz, A., Paganetto, G., Botti, G. and Pavan, B. Cancer stem cells and nanomedicine: new opportunities to combat multidrug resistance?. *Drug Discovery Today*, **25**(9), 1651-1667(2020). https://doi.org/10.1016/j.drudis.2020.07.023

22. Fan, Y. J. and Zong, W. X. The cellular decision between apoptosis and autophagy. *Chinese Journal of Cancer,* **32**(3), 121–129 (2013). https://doi.org/10.5732/cjc.012.10106

23. Yu, G. and Klionsky, D. J. Life and Death Decisions—The Many Faces of Autophagy in Cell Survival and Cell Death. *Biomolecules,* **12**(7), 866(2022). https://doi.org/10.3390/biom12070866.

24. Fu, X., Li, M., Tang, C., Huang, Z. and Najafi, M. Targeting of cancer cell death mechanisms by resveratrol: a review. *Apoptosis,* 1-13(2021). https://doi.org/10.1007/s10495-021-01689-7

25. Li, J., Fan, Y., Zhang, Y., Liu, Y., Yu, Y. and Ma, M. Resveratrol Induces Autophagy and Apoptosis in Non-Small-Cell Lung Cancer Cells by Activating the NGFR-AMPK-mTOR Pathway. *Nutrients,* **14**(12), 2413(2022). https://doi.org/10.3390/nu14122413

26. Lossi, L. The concept of intrinsic versus extrinsic apoptosis. *Biochemical Journal,* **479**(3), 357-384(2022). https://doi.org/10.1042/BCJ20210854.

27. Neophytou, C. M., Trougakos, I. P., Erin, N. and Papageorgis, P. Apoptosis Deregulation and the Development of Cancer Multi-Drug Resistance. *Cancers,* **13**(17), 4363(2021). https://doi.org/10.3390/cancers13174363

28. Xu, Y. and Ye, H. Progress in understanding the mechanisms of resistance to BCL-2 inhibitors. Experimental *Hematology & Oncology,* **11**(1), 31(2022). https://doi.org/10.1016/S1097-2765(01)00320-3

29. Roshani, M., Jafari, A., Loghman, A., Sheida, A. H., Taghavi, T., Zadeh, S. S. T. and Mirzaei, H. Applications of resveratrol in the treatment of gastrointestinal cancer. *Biomedicine & Pharmacotherapy*, **153,** 113274(2022). https://doi.org/10.1016/j.biopha.2022.113274

30. Biswas, P., Dey, D., Biswas, P. K., Rahaman, T. I., Saha, S., Parvez, A. and Kim, B. A comprehensive analysis and anti-cancer activities of quercetin in ROS-mediated cancer and cancer stem cells. *International Journal of Molecular Sciences,* **23**(19), 11746(2022). https://doi.org/10.3390/ijms231911746.

31. Сапарбекова, А. А. Extraction of resveratrol from the pomace of various grape varieties using an enzyme preparation. Вестник Евразийского национального университета имени ЛН Гумилева. *Серия Биологические науки,* **139**(2), 6-14 (2022). https://bulbio.enu.kz/index.php/main/article/view/369.

32. Al-Jubouri, E. S. J., Abas, W. F. and Al-Mousawi, A. J. Extraction and Purification of Resveratrol from Grape Waste. *Indian Journal of Forensic Medicine & Toxicology,* **16**(1),455 (2022).

33. Md Toha, Z., Haron, N. H., Kamal, N. N. S. N. M., Khairuddean, M. and Arsad, H. Exploring Clinacanthus nutans leaf different solvent extracts on antiproliferative effects induced metastasis through apoptosis and cell cycle against MCF-7 human breast cancer cell lines. *Future Journal of Pharmaceutical Sciences,* **8**(1), 1-16(2022).

34. Wang, B., Moon, S. P., Cutolo, G., Javed, A., Ahn, B. S., Ryu, A. H. and Pratt, M. R. HSP27 Inhibitory Activity against Caspase-3 Cleavage and Activation by Caspase-9 Is Enhanced by Chaperone O-GlcNAc Modification in Vitro. *ACS Chemical Biology,* **18**(8), 1698-1704(2023). https://doi.org/10.1021/acschembio.3c00270.

35. Kim, Y. A., Choi, B. T., Lee, Y. T., Park, D. I., Rhee, S. H., Park, K. Y. and Choi, Y. H. Resveratrol inhibits cell proliferation and induces apoptosis of human breast carcinoma MCF-7 cells. *Oncology Reports,* **11**(2), 441–446(2004).

36. Ko, J. H., Sethi, G., Um, J. Y., Shanmugam, M. K., Arfuso, F., Kumar, A. P., Bishayee, A. and Ahn, K. S. The Role of Resveratrol in Cancer Therapy. *International Journal of Molecular Sciences,* **18**(12), 2589(2017). https://doi.org/10.3390/ijms18122589

37. Scarlatti, F., Maffei, R., Beau, I., Codogno, P. and Ghidoni, R. Role of non-canonical Beclin 1-independent autophagy in cell death induced by resveratrol in human breast cancer cells. *Cell Death & Differentiation*, **15**(8), 1318-1329(2008).

38. Prerna, K. and Dubey, V. K. Beclin1-mediated interplay between autophagy and apoptosis: New understanding. *International Journal of Biological Macromolecules,* **204**, 258-273(2022). https://doi.org/10.1016/j.ijbiomac.2022.02.005

39. Sakalli-Tecim, E., Gur-Dedeoglu, B. and Guray, N. T. Systems biology based miRNA–mRNA expression pattern analysis of Emodin in breast cancer cell lines. *Pathology-Research and Practice,* **249**, 154780(2023). https://doi.org/10.1016/j.prp.2023.154780

40. Kowsari, H., Davoodvandi, A., Dashti, F., Mirazimi, S. M. A., Bahabadi, Z. R., Aschner, M. and Mirzaei, H. Resveratrol in cancer treatment with a focus on breast cancer. *Current Molecular Pharmacology*, **16**(3), 346-361(2023). https://doi.org/10.2174/1874467215666220616145216

41. Hussain, A. R., Uddin, S., Bu, R., Khan, O. S., Ahmed, S. O., Ahmed, M. and Al-Kuraya, K. S. Resveratrol suppresses constitutive activation of AKT via generation of ROS and induces apoptosis in diffuse large B cell lymphoma cell lines. *PloS one,* **6**(9), e24703(2011). https://doi.org/10.1371/journal.pone.0024703

42. Chen, Q., Ganapathy, S., Singh, K. P., Shankar, S. and Srivastava, R. K. Resveratrol induces growth arrest and apoptosis through activation of FOXO transcription factors in prostate cancer cells. *PloS one,* **5**(12), e15288(2010). https://doi.org/10.1371/journal.pone.0015288

43. Jiang, Q., Yang, M., Qu, Z., Zhou, J. and Zhang, Q. Resveratrol enhances anticancer effects of paclitaxel in HepG2 human liver cancer cells. *BMC Complementary and Alternative Medicine,* **17**(1), 477(2017). https://doi.org/10.1186/s12906-017-1956-0

44. Kello, M., Takac, P., Kubatka, P., Kuruc, T., Petrova, K. and Mojzis, J. Oxidative Stress-Induced DNA Damage and Apoptosis in Clove Buds-Treated MCF-7 Cells. *Biomolecules,* **10**(1), 139(2020). https://doi.org/10.3390/biom10010139

45. Chan, Wen-Hsiung. The Signaling Cascades of Ginkgolide B-Induced Apoptosis in MCF-7 Breast Cancer Cells. *International Journal of Molecular Sciences*, **8**(11), 1177-1195(2007). https://doi.org/10.3390/i8111177

46. Micheli, L., Testai, L., Angeli, A., Carrino, D., Pacini, A., Margiotta, F., Flori, L., Supuran, C. T., Calderone, V., Ghelardini, C. and Di Cesare Mannelli, L. Inhibitors of Mitochondrial Human Carbonic Anhydrases VA and VB as a Therapeutic Strategy against Paclitaxel-Induced Neuropathic Pain in Mice. *International Journal of Molecular Sciences,* **23** (11), 6229(2022).https://doi.org/10.3390/ijms23116229

47. Braicu, C., Zanoaga, O., Zimta, A. A., Tigu, A. B., Kilpatrick, K. L., Bishayee, A. and Berindan-Neagoe, I. Natural compounds modulate the crosstalk between apoptosis-and autophagy-regulated signaling pathways: Controlling the uncontrolled expansion of tumor cells. *In Seminars in Cancer Biology,* **80**, 218-236(2022). Academic Press (2022, May). https://doi.org/10.1016/j.semcancer.2020.05.015

48. Ulakcsai, Z., Bagaméry, F., Vincze, I., Szökő, É. and Tábi, T. Protective effect of resveratrol against caspase 3 activation in primary mouse fibroblasts. *Croatian Medical Journal,* **56**(2), 78–84(2015). https://doi.org/10.3325/cmj.2015.56.78

49. Jang, J. Y., Im, E. and Kim, N. D. Mechanism of Resveratrol-Induced Programmed Cell Death and New Drug Discovery against Cancer: A Review. *International Journal of Molecular Sciences,* **23**(22), 13689(2022). https://doi.org/10.3390/ijms232213689

50. Al-Odat, O. S., Guirguis, D. A., Schmalbach, N. K., Yao, G., Budak-Alpdogan, T., Jonnalagadda, S. C. and Pandey, M. K. Autophagy and Apoptosis: Current Challenges of Treatment and Drug Resistance in Multiple Myeloma. *International Journal of Molecular Sciences*, **24**(1), 644(2022).

https://doi.org/10.3390/ijms24010644

51. Ashrafizadeh, M., Taeb, S., Haghi-Aminjan, H., Afrashi, S., Moloudi, K., Musa, A. E. and Farhood, B. Resveratrol as an enhancer of apoptosis in cancer: a mechanistic review. *Anti-Cancer Agents in Medicinal Chemistry* (Formerly *Current Medicinal Chemistry-Anti-Cancer Agents)*, **21**(17), 2327-2336(2021). https://doi.org/10.2174/1871520620666201020160348

52. Mohammed, I.H., Hameed, A.T. and Salman, H.F. Phytochemical and biological of Anthemis nobilis (Asteraceae family) a native herbs of Iraq. *Systematic Reviews in Pharmacy,* **11**(2), 458–461 (2020).

53. Issa, N.I., Mohammed Hasan, Z.Y. and Hameed, A.T. Phytochemical investigation and antioxidant activity of total phenols in the aerial parts of some Asteraceae family wild plants grown in western of Iraq. *Systematic Reviews in Pharmacy,* **11**(1), 62–68 (2020).

54. Asep, S., Sutyarso, S., Hendri, B., Nuning, N. and Evi, K. Antioxidant and Cytotoxic Activities of Melinjo (Gnetum gnemon L.) Seed Fractions on HeLa Cell Line an In Vitro. *Pharmacognosy Journal,* **14**(3),559-564 (2022).

55. Brockmueller, A., Buhrmann, C., Shayan, P. and Shakibaei, M. Resveratrol induces apoptosis by modulating the reciprocal crosstalk between p53 and Sirt-1 in the CRC tumor microenvironment. *Frontiers in Immunology,* **14**, pp 1-14(2023). DOI 10.3389/fimmu.2023.1225530

56. Al-Musawi, A.H., Aliwy, S.A. and Al-Azerg, L.K.A. Isolate two species of compositae family E. armatus Boiss & Hausskn and E. cephalotes DC. by morphological and vegetative shoot apex for the first time in Iraq. *Iraqi Journal of Agricultural Sciences,* **48** (5), 1247–1254 (2017).

ريسفيراترول يمنع ديناميكيات دورة الخلية، وتنشيط كاسباس، وموت الخلايا المبرمج:

الآثار المترتبة على علاج السرطان في خلايا MCF-7 "

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ريسفيراترول هو بوليفينول مشتق من النبات معروف بخصائصه المضادة للالتهابات والمضادة للأورام في النماذج المختبرية والكائنات الحية. تشير الدراسات الحديثة إلى أن بعض نظائرها من ريسفيراترول قد تظهر عوامل أكثر فعالية مضادة للورم. تبحث هذه الدراسة في تأثير الريسفيراترول على الدورة الخلوية لخلايا MCF-7 بتركيزين 1.5 و 2 ميكروغرام/ مل. ريسفيراترول، عند 2 ميكروجرام/مل، يغير توزيع دورة الخلية بشكل ملحوظ، مما يشير إلى تأثير يعتمد على التركيز. يعمل دوكسوروبيسين كعنصر تحكم إيجابي ويحث على إيقاف المرحلة G1. تساهم هذه النتائج في فهم الآليات المحتملة للريسفيراترول في علاج السرطان وتسليط الضوء على تأثيره على ديناميات دورة الخلية. بالإضافة إلى ذلك، تستكشف الدراسة آثار تركيزات مختلفة من ريسفيراترول على نشاط كاسباس 8، وذلك باستخدام دوكسوروبيسين كعنصر تحكم إيجابي للمقارنة. تم تعريض خطوط الخلايا البشرية لتركيزات متفاوتة من الريسفيراترول (1.5 ميكروجرام/مل و 2 ميكروجرام/مل)، وتمت مقارنة أنشطة كاسبيز 8 مع كل من الخلايا غير المعالجة (المجموعة الضابطة) والخلايا المعالجة بالدوكسوروبيسين. تم حساب متوسط نشاط Caspase 8 والانحراف المعياري لكل مجموعة علاجية، وتم تحديد الأهمية الإحصائية. توفر دراستنا مزيدًا من التبصر حول خصائص ريسفيراترول المضادة للسرطان في خلايا الثدي في المختبر.

الكلمات الدالة: ريسفيراترول، ديناميات دورة الخلية، تفعيل كاسباس، خلايا MCF-7، دوكسوروبيسين، موت الخلايا المبرمج، غشاء الميتوكوندريا.