Cytotoxic Effect of Resveratrol on Colorectal Cancer Cell Line

Hussein A. Khayoon[1*] and Falah M K Al-Rekabi[2]

[1]Department of Basic Sciences, College of Nursing, Al-Muthanna University, [2]Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Iraq

ABSTRACT

This study aimed to examine the cytotoxic effect of resveratrol as an anticancer in human colorectal cancer (HRT) cell line by assessment of its half-maximal inhibitory concentration (IC_{50}) and its ability to inhibit the growth of these cancerous cells. Resveratrol inhibited the proliferation of HRT cell lines when used at different increased concentrations in this study (25, 50, 100, 200, and 300 µmol). These increased concentrations of resveratrol caused a corresponding significant inhibition in the growth percentage of the tested cancerous cell line (13%, 31.33%, 53.66%, 63.66 %, and 76.33%, respectively) when compared with DMSO0.1% as negative control, in a concentration-dependent manner. Resveratrol at 300 µmol concentration showed the highest significant increase in the growth inhibitory percentage (76.33%). Moreover, resveratrol IC_{50} against HRT cell line was determined as 75.63 µmol. The study suggests a promising anticancer activity of resveratrol, which can interfere with many dysregulated signaling pathways in transformed cells which are proposed to be driving forces for its anticancer effect.

Keywords: Resveratrol, Anti-colorectal cancer, Cancer cell line

Introduction

Colorectal cancer (CRC) is the third most diagnosed cancer worldwide and ranks second among cancer-related deaths in many countries (1). The proportions of newly diagnosed CRC cases are 9.2% in men and 10% in women, which accounts for 9.7% of overall cancer incidence worldwide (2). The risk of CRC increases with age, hereditary genetic predisposition, chronic bowel inflammation, and diabetes, in addition, smoking, alcohol consumption, low vegetable intake, obesity, and sedentary lifestyle are associated with an increased incidence of CRC (3).

In contrast, chronic intake of certain non-steroidal anti-inflammatory drugs, high calcium consumption, and post-menopausal hormone usage are associated with reduced risk of the disease (4). About 70% of CRC occurs without familial or inherited predisposition (sporadic disease), while less than 10% of patients inherit the tendency to develop the disease (5). The most common inherited conditions that predispose to CRC are familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome). FAP accounts for less than 1% of all CRC cases and is associated with autosomal dominant inheritance of a mutant adenomatous polyposis coli (*Apc*) gene. FAP patients develop hundreds of colonic adenomatous polyps that start malignant transformation when the patient is as young as 20 and almost all patients develop cancer by the age of 40 years unless the colon is removed. HNPCC is an autosomal dominant disease that accounts for 2-3% of all CRC cases. It is associated with alterations in human mutL homolog 1*(MLH1)* and MutS homolog 2*(MSH2*) genes that are important in DNA repair systems, thus they may also be associated with other malignancies such as cancer of the pancreas, kidney, stomach, ovary, and endometrium (6). Unlike FAP, HNPCC patients develop a few adenomas with 70-80% malignant

*Correspondence: h.shark@yahoo.com
Department of Basic Sciences, College of Nursing, Al-Muthanna University. Received: 27 November 2019, Accepted: 7 April 2020, Published: 28 June 2020.

DOI: https://doi.org/10.30539/ijvm.v44i1.939

transformation rate, and are usually diagnosed in their mid-forties (7). In addition, germline mutations of the liver kinase B1/Serine/threonine kinase 11*(LKB1/STK11*) gene are associated with Peutz– Jeghers syndrome and mutations of small mother cell against decapentaplegic *(SMAD4*) or activing like kinase 3*(ALK3)* result in juvenile polyposis (8).

Resveratrol (3,5,4'-trihydroxy-*trans*-stilbene). is a polyphenolic natural product which belongs to the group of phytoalexins that are synthesized when plants are subject to environmental stress, such as exposure to pathogens. It can be found in widely consumed foods and beverages such as red wine, grapes, peanuts, pistachios, berries, and Itadori tea. In fact, resveratrol is produced by more than 70 species of plants in response to stress. Resveratrol is particularly thought to mediate some of the potential health benefits of red wine (9).

Many studies have examined and described different actions of resveratrol, such as antioxidant and anti-inflammatory properties. For instance, the serum antioxidant activity is shown to be significantly elevated by up to 18% in normal individuals after ingestion of 300 ml of red wine (10). In addition, moderate intake of red wine has been revealed to influence by decrease level of low-density lipoprotein (LDL) in humans (11). Resveratrol has been shown to exert effects similar to dietary restriction, increasing the lifespan of animal experimental models (12). Moreover, resveratrol can act as an inhibitor of the pro-inflammatory cyclooxigenase-2 COX-2 enzyme, in addition to its inhibitory effect on human platelet aggregation and eicosanoid synthesis (13). The effects of resveratrol appear to be achieved through numerous cell signaling pathways, including induction of cell cycle arrest, stimulation of apoptosis and differentiation, suppression of inflammation and angiogenesis, and reduction of adhesion, invasion, and metastasis (14). Various *in vitro* studies have described antioxidant, anti-inflammatory, and anticancer features of resveratrol, exerted through modification of signaling pathways such as downregulating WNT2 and reducing the nuclear accumulation of β-catenin and transcription factors involved in cellular processes such as apoptosis, stimulation of cell cycle arrest and lipid oxidation (15). Different studies have explained the effects of resveratrol in several human colorectal cell lines and the related mechanisms. For example, in the human colonic adenocarcinoma cell line Caco2 and the HCT-116 colon carcinoma cell line, resveratrol was revealed as a cell cycle inhibitor, indicated by low cyclin D1 and cdk4, and as an apoptosis inducer, shown by high caspase activity (16). Regarding the resveratrol significant biological role as cell cycle inhibitor, the current study was designated for detection its *in vitro* cytoxicity on cancer cell line as preliminary for future *in vivo* studies.

Materials and Methods

Maintenance of Cell Cultures

Homo sapiens, human colon tissue (HRT cells) were provided by Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR), Baghdad, Iraq, and maintained in Roswell Park Memorial Institute medium (RPMI-1640) (S,p.A., Italia) supplemented with 10% fetal calf serum (Capricorn, Germany). These are important because they act as growth factors, which facilitate cell survival, and proliferation, 100 units/ml of penicillin, and 100 µg/mL of streptomycin were added to the medium. Cells were passaged using Trypsin-EDTA (Ethylenediaminetetraacetic acid) (Capricorn, Germany) reseeded at 80% confluence twice a week, and incubated in CO_2 incubator (Bio Tek, USA) 5% CO_2 at 37 °C (17).

Cytotoxicity Assays

To determine the cytotoxic effect of resveratrol, the Methyl thiazolyl tetrazolium (MTT) cell viability assay was done using 96-well plates. Cell lines were seeded at 1×10^4 cells/well. After 24 h, or a confluent monolayer was achieved, cells were treated with resveratrol at different concentrations (25, 50, 100, 200, and 300 µmol). Cell viability was measured after 72 h of treatment by removing the medium, adding 28 µL of 2 mg/mL solution of MTT stain and incubating the cells for 3 h at 37 °C. After removing the MTT solution, the crystals remained in the wells were solubilized by the addition of 130 µL of DMSO 2.5% (Dimethyl Sulphoxide) followed by 37 °C in CO_2 incubator (Cypress Diagnostics, Belgium) at 5% of CO_2 for 15 min with shaking (18). The absorbency was determined on a micro plate

reader (Bio Tek, USA) at 492 nm, the assay was performed in triplicate. The inhibition rate of cell growth (the percentage of cytotoxicity) was calculated as the following equation:

Cytotoxicity = A-B/A *100%

Where A is the optical density of control and B the optical density of samples.

Materials and Chemicals

Trypsin-EDTA (0.5%) was purchased from Capricorn (Germany) causes cells to detach from the growth surface. Methyl thiazolyl tetrazolium (MTT) solution: was purchased from Sigma-Aldrich Co. Fetal calf serum was purchased from Capricorn (Germany). These are important because the growth factors facilitate cell survival and proliferation while antibodies could bind to the cells in culture. RPMI 1640 was purchased from S. p. A. (Italy) as a supplement media. The cell lines used in this study were supplied by tissue culture unit, Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR) Baghdad, Iraq maintained in RPMI-1640. Resveratrol with chemical formula $C_{14}H_{12}O_3$ g/mol. resveratrol purity 99% was purchased from SIGMA and ALDRICH. Resveratrol was dissolved in DMSO 0.1% as stock and stored at -20 °C. Stock solution of resveratrol (1000 µmol) was prepared in DMSO 0.1% and then five different concentrations were prepared (25, 50, 100, 200, and 300 µmol).

Statistical Analysis

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 6. The values were presented as the mean±SEM of triplicate measurements (19).

Results and Discussion

The result showed that the IC_{50} of resveratrol on HRT colon cancer cell line is 75.63 µmol at 72 h of incubation. Growth inhibition of HRT cell line listed in Figure 1 and Table 1, showed positively proportional concentration-dependent effect of resveratrol (25, 50, 100, 200, and 300 µmol) that caused significant ($P \leq 0.05$) growth inhibition percentage of 13%, 31.33%, 53.66%, 63.66 %, and 76.33%, respectively, for resveratrol when compared with DMSO (0.1%) as the vehicle control, where the growth of HRT cell line was 100%.

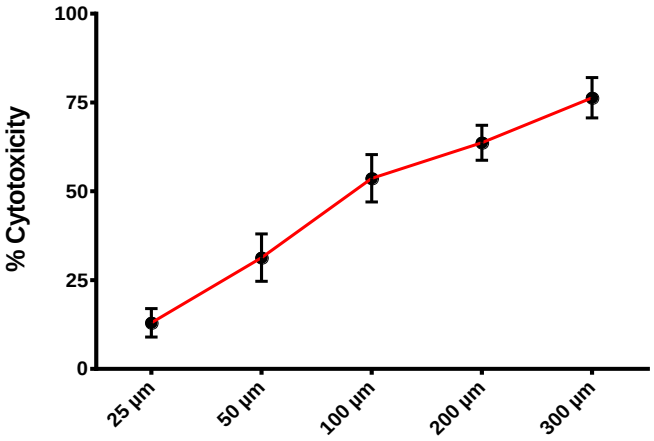

Figure 1. Cytotoxicity effect of resveratrol in HRT cells. IC_{50}= 75.63 µmol

Table 1. Growth inhibition effect of resveratrol in different concentrations in HRT colon cancer cell line after 72 h exposure

No.	Resveratrol concentrations per µmol at 72 h	Growth inhibition percentages of HRT cells
1-	25 µmol	13%
2-	50 µmol	31.33%
3-	100 µmol	53.66%
4-	200 µmol	63.66%
5-	300 µmol	76.33%

Current treatments for colon cancer are only effective in 50% of the cases (20). In addition, these treatments (chemotherapy and radiation) are frequently toxic to normal host cells (21). Many plant-derived phytochemicals have been shown to be beneficial to human health like resveratrol and catechin compounds which were tested previously against each of four human tumor cell lines (MCF-7 breast carcinoma, HT-29 colon carcinoma, A-427 lung carcinoma and UACC-375 melanoma (22). In the present study, we sought to determine the effect of resveratrol on cancer cell growth. Anti-proliferative effect of resveratrol was analyzed using a colon cancer cell line.

Current results of the inhibitory effect of different resveratrol concentrations against *in vitro* growth

of (HRT cell line) showed dose dependent inhibitory cancer growth for all concentrations used in this study against HRTcell line. The maximum effect of inhibiting cancer cell growth was seen with the concentration of (300) µmol of resveratrol. We speculate that this inhibitory effect of resveratrol might be due to many reasons. The first one is that it may be the mechanism of anti-oxidative or potent free radical scavenging effects of resveratrol and possibly other reported mechanisms like receptor mediated inhibitory which interfere and inhibit cancer cell growth (23). Other mechanisms could be hypermethylation or induction of suppressor gene, apoptosis, DNA repair or increasing cellular gap junction. Interestingly results of previous published study indicated that resveratrol causes apoptosis and mitochondrial dysfunction by membrane depolarization in malignant human pancreatic cancer cell (24). Resveratrol has protective effects against lung cancer (25) and many more anti-cancer reports are present in the literature that strongly suggest a specific cytotoxic effect on cancerous rather than non-cancerous cells. These results suggest that resveratrol can induce cell cycle arrest and apoptosis independently of p53 status (26). Nevertheless, other researchers have reported that p53 upregulation could play an important role on the synergistic effect between resveratrol and etoposide, a topoisomerase II inhibitor used as an antineoplastic drug (27).

Other researches showed that resveratrol also sensitizes HT-29 and SW620 colorectal cancer cell lines to cytotoxic oxidative stress induced by 5-flourouuracil 5-FU, by inhibiting their endogenous antioxidant capacity (28). Moderate resveratrol concentration (15 µmol) in combination with very low 5-FU (0.5 µmol) concentration causes significant inhibition of cell proliferation, migration, and cell cycle arrest at S phase, leading to apoptosis in HCT-116 cells. The same study provides evidence suggesting that its mechanism of action may be related with the activation of the MAPK pathway through upregulation of p-JNK and p38, with no p-ERK changes (29). Similar results were found in a study with etoposide resistant HT-29 cells, where resveratrol was able to chemosensitize HT-29 cells promoting cell cycle inhibition, reactive oxygen species (ROS) generation, AMPK activation, and apoptosis induction (30).

In conclusion, CRC is a prevalent cancer and one of the main causes of cancer mortality entire the world. Several factors from genetics to diet are involved in the incidence of this malignancy. Its pathophysiology is heterogeneous which multiple molecules and various signaling pathways including inflammation, oxidative stress, and apoptosis are implicated in its incidence and progression. Several studies have supported the potential effects of resveratrol in CRC treatment. This polyphenol compound represents different properties including antioxidant, anti-inflammatory, apoptosis inducer, and anti-angiogenesis efficacy. Due to these significant effects, resveratrol is suggested as a novel therapeutic agent for cancers. Moreover, some studies reported that consumption of resveratrol in combination with other anti-cancer drugs can increase their effects and decrease their side effects. Thus, this multi-tasking compound can be suggested as a new candidate in CRC treatment however, more human studies are needed.

Acknowledgments

The authors are greatly appreciable to Iraqi Centre for Cancer and Medical Genetics Research (ICCMGR) Baghdad, Iraq for supplying HRT cells.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Lee SY, Haq F, Kim D, Jun C Jo HJ, Ahn SM, *et al.* Comparative genomic analysis of primary and synchronous metastatic colorectal cancers. PloS One. 2014; 5,9 (3): 59-90.
2. Dušek L, Mužík J, Malúšková D, Šnajdrová L. Epidemiology of colorectal cancer: international comparison [online]. Institute of Biostatistics and Analyses, Faculty of Medicine, Masaryk University, Brno Czech Republic.2015;1,1402.
3. Rasool S, Rasool V, Naqvi T, Ganai BA, Shah BA. Genetic unraveling of colorectal cancer. Tumour Biology: Tumour Biol. 2014;17(5):1-4.

4. Steward WP, Brown K. Cancer chemoprevention: a rapidly evolving field.Br. J. Cancer. 2013; 109: 1-7.
5. Hasanpour M, Galehdari H, Masjedizadeh A, Ajami N. A unique profile of adenomatous polyposis coli gene mutations in Iranian patients suffering sporadic colorectal cancer. Cell J.2014; 16: 17-24.
6. Steinke V, Engel C, Buttner R, Schackert HK, Schmiegel WH, Propping P. Hereditary nonpolyposis colorectal cancer (HNPCC)/Lynch syndrome. DTSCH ARZTEBL INT. 2013; 110: 32-38.
7. Fostira F, Thodi G, Konstantopoulou I, Sandaltzopoulos R, Yannoukakos D. Hereditary cancer syndromes. Journal of B.U.ON.2007;1: 13-22.
8. Wang Z, Chen Y, Wu B, Zheng H, He J, Jiang B. A novel mutation in STK11 gene is associated with Peutz-Jeghers syndrome in Chinese patients. BMCMEDGENET. 2011; 12:12-161.
9. Mishra RN. Resveratrol -the new rasayan (antiaging) drug. BMC Complement Med Ther.2011; 1: 5-18.
10. Whitehead TP, Robinson D, Allaway S, Syms J, Hale A. Effect of red wine ingestion on the antioxidant capacity of serum. ClinChem Lab Med.1995; 41: 32-35.
11. Blanco-Colio LM, Valderrama M, Alvarez-Sala LA, Bustos C, Ortego M, Hernandez-Presa MA, *et al.* Red wine intake prevents nuclear factor-kappaB activation in peripheral blood mononuclear cells of healthy volunteers during postprandial lipemia. Circulation. pubmed.2000; 102: 1020-1026.
12. Baur JA, Sinclair DA. Therapeutic potential of resveratrol: the *in vivo* evidence. Nat Rev Drug Discov.2006; 5: 493-506.
13. Bode LM, Bunzel D, Huch M, Cho GS, Ruhland D, Bunzel M. *et al.* In vivo and in vitro metabolism of trans-resveratrol by human gut microbiota. Am J ClinNutr.2013; 97: 295-309.
14. Storniolo CE, Moreno JJ. Resveratrol metabolites have an antiproliferative effect on intestinal epithelial cancer cells. Food Chem X.2012; 134: 1385-1391.
15. Tomé-Carneiro J, Larrosa M, Gonzalez-Sarrias A, Tomas-Barberan FA, Garcia-Conesa MT, Espin JC. Resveratrol and clinical trials: the crossroad from in vitro studies to human evidence. Current Pharmaceutical Design.2013; 19: 6064-6093.
16. Wolter F, Akoglu B, Clausnitzer A, Stein J. Downregulation of the cyclin D1/Cdk4 complex occurs during resveratrol-induced cell cycle arrest in colon cancer cell lines. J ClinNutr Food Sci. 2001; 131: 2197-2203.
17. Sulaiman GM, Jabir MS, Hameed AH. Nanoscale modification of chrysin for improved of therapeutic efficiency and cytotoxicity. Arificial Artif Cells Nanomed Biotechnol. 2018;1: 1-8.
18. Jabir MS, Taha AA, Sahib UI, Taqi ZJ, Al-Shammari AM, Salman AS. Novel of nano delivery system for linalool loaded on gold nanoparticles conjugated with CALNN peptide for application in drug uptake and induction of cell death on breast cancer cell line, Mater. Sci. Eng. C.2019; 94:949-964.
19. Jabir MS, Sulaiman GM, Taqi ZJ, Li D. Iraqi propolis increases degradation of IL-1β and NLRC4 by autophagy following Pseudomonas aeruginosa infection, Microb Infect.2018;20: 89-100.
20. Longe JL. The Gale Encyclopedia of Medicine. 3rd ed Thompson Gale Corporation 2006; 2:939.
21. Geller E, Editors. Encyclopedia of Science and Technology. New York: McGraw-Hill. 2020. 3:424p.
22. Manhal FA . Cytotoxic effect of green tea leaf extract on tumor cell line. Iraqi .J.Vet.Med.2017; 41(1):71-75.
23. Tripodo G, Mandracchia D, Collina S, Rui M, Rossi D. New perspectives in cancer therapy: the biotin-antitumor molecule conjugates. Med chem, an open access. 2014; S1(004):1-8.
24. Sun W, Wang W, Kim J. Anti-cancer effect of resveratrol is associated with induction of apoptosis via a mitochondrial pathway alignment. Adv Exp Med Biol. 2008; 614:179-86.
25. Whyte L, Huang YY, Torres K, Mehta RG. Molecular mechanism of resveratrol action in lung cancer cells using dual protein and microarray analyses. Cancer Res. 2007; 67:12007-17.
26. Fulda S, Debatin KM. Sensitization for anticancer drug-induced apoptosis by the chemopreventive agent resveratrol. Oncogene.2004;23: 6702-6711.

27. Amiri F, Zarnani AH, Zand H, Koohdani F, Jeddi-Tehrani M, Vafa M. Synergistic anti-proliferative effect of resveratrol and etoposide on human hepatocellular and colon cancer cell lines. Eur. J. Pharmacol. 2013; 718(1-3): 34-40.
28. Santandreu F M, Valle A, Oliver J, Roca P. Resveratrol potentiates the cytotoxic oxidative stress induced by chemotherapy in human colon cancer cells. Cell. Physiol. Biochem. 2011; 28: 219-228.
29. Mohapatra P, Preet R, Choudhuri M, Choudhuri T, Kundu C N. 5-fluorouracil increases the chemopreventive potentials of resveratrol through DNA damage and MAPK signaling pathway in human colorectal cancer cells. Oncol. Re.2011; 19: 311–321.
30. Hwang JT, Kwak D W, Lin S K, Kim H M, Kim YM, Park OJ. Resveratrol induces apoptosis in chemoresistant cancer cells via modulation of AMPK signaling pathway. Ann. N.Y. Acad. Sci. 2007;1095: 441-448.

التأثير السمي الخلوي لماده الرزفيراترول على خط الخلايا السرطانيةللقولون والمستقيم

حسين علي خيون[1] و فلاح موسى كاظم الركابي [2]

[1]فرع العلوم الاساسيه, كلية التمريض, جامعه المثنى.[2] فرع الفسلجه والكيمياء الحياتية والادويه, كلية الطب البيطري ,جامعه بغداد.

ألخلاصة

هدفت هذه الدراسة لفحص التأثير السام للخلايا باستخدام الريسفيراترول كمضاد للسرطان في خط خلايا سرطان القولون والمستقيم البشري (HRT) من خلال تقييم تركيزه النصف مثبط (IC50) وقدرته على تثبيط نمو هذه الخلايا السرطانية. تم الكشف عن التأثير التثبيطي للريسفيراترول على خطوط الخلايا HRT عند استخدامه بتراكيز متزايدة مختلفة في هذه الدراسة (25 ، 50 ، 100 ، 200 ، 300) ميكرومول على التوالي. تسببت هذه التراكيز المتزايدة من الريسفيراترول في تثبيط كبير مناظر في نسبة نمو خط الخلايا السرطانية المعالجة (13٪ و 31.33٪ و 53.66٪ و 63.66٪ و 76.33٪) على التوالي عند مقارنتها بـ 0.1 DMSO٪ كعنصر تحكم سلبي و بطريقة تعتمد على التركيز. أظهر الريسفيراترول بتركيز 300 ميكرومول أعلى زيادة معنوية في نسبة مثبطات النمو (76.33٪). علاوة على ذلك ، تم تحديد ريسفيراترول IC50 مقابل خط خلية (HRT) على أنه 75.63 ميكرومول. اقترحت الدراسة نشاطًا واعدًا ضد سرطان القولون والمستقيم باستخدام للريسفيراترول والذي يمكن أن يتداخل مع العديد من مسارات الإشارات غير المنظمة الخاصة بالخلايا المتغيرة والتي يُقترح أن تكون قوى دافعة لتأثيرها المضاد للسرطان.

الكلمات المفتاحية:ريسفيراترول،سرطان القولون والمستقيم،خط الخلايا السرطانية