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ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE EFFECTS OF ORCINOL ON PROLIFERATION AND APOPTOSIS OF SW480 HUMAN COLORECTAL CANCER CELLS

ORSİNOL'ÜN SW480 İNSAN KOLOREKTAL KANSER HÜCRELERİNDE PROLİFERASYON VE APOPTOZ ÜZERİNE ETKİLERİ

Betul YANIK[1,2], Filiz BAKAR-ATES[2]*

[1]Ankara University, Institute of Health Sciences, 06110, Ankara, Turkey

[2]Ankara University, Faculty of Pharmacy, Department of Biochemistry, 06560, Ankara, Turkey

ABSTRACT

Objective: *Colorectal cancer is a rapidly increasing disease worldwide, and almost half of the diagnosed patients die from this disease each year. The methods used in the treatment of colorectal cancer, including traditional treatment methods such as surgery, radiotherapy and current chemotherapy options, have low effectiveness and have many side effects. Because of all these problems, the importance of developing new agents for the treatment of colorectal cancer is increasing. Orsinol is a secondary metabolite isolated from lichens, and there are findings regarding the antioxidant, antimicrobial and antidepressant activity of the compound. In recent years, research on the anticancer activity of the compound has also started to take place in the literature. In this study, it was aimed to investigate the efficacy of orcinol on cell proliferation and apoptosis in human SW480 colorectal cancer cells.*

Material and Method: *Within the scope of the study, SW480 human colorectal cancer cells were used and cultured in DMEM medium. Orcinol was dissolved with dimethylsulfoxide to prepare a stock solution and applied to the cells in a concentration range of 1-25 mM. The effect of orcinol on cell viability was determined by MTT test. The apoptotic activity of the compound was evaluated with Annexin V binding assay using the Muse Cell Analyzer.*

Result and Discussion: *The results of MTT analysis showed that orcinol significantly decreased cell viability at 5 mM and above ($p<0.05$). While cell viability was 100.00±6.14% in the control group, it was determined as 12.50±0.65% in cells treated with 25 mM orcinol ($p<0.0001$). According to Annexin V binding analysis findings, the early apoptotic cell population was 12.06±1.22% in the 25 mM orcinol treated group, while it was 0.60±0.11% in the control group. The findings obtained from the study showed that that orcinol has a cytotoxic effect at high concentration on SW480 colorectal cancer cells, and further studies are needed to increase the efficiency of the compound and to elucidate its mechanism of action.*

* **Corresponding Author / Sorumlu Yazar:** Filiz Bakar-Ates
e-mail / e-posta: fbakar@ankara.edu.tr, **Phone / Tel.:** +903122033055

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ÖZ

Amaç: *Kolorektal kanser, her yıl dünya çapında hızla artan bir hastalıktır. Teşhis konulmuş hastaların neredeyse yarısı her yıl bu hastalıktan hayatını kaybetmektedir. Kolorektal kanserin tedavisi için uygulanan geleneksel tedavi yöntemleri olan cerrahi, radyoterapi ve mevcut kemoterapi seçenekleri de dahil olmak üzere tedavide kullanılan yöntemlerin etkinliği düşüktür ve çok fazla yan etkileri bulunmaktadır. Tüm bu problemler nedeni ile kolorektal kanserin tedavisi için yeni ajanlar geliştirmenin önemi giderek artmaktadır. Orsinol, likenlerden izole edilen bir sekonder metabolit olup, söz konusu bileşiğin antioksidan, antimikrobiyal ve antidepresan aktivitesine yönelik bulgular mevcuttur. Son yıllarda bileşiğin antikanser etkinliğine dair araştırmalar da literatürde yer almaya başlamıştır. Bu çalışmada, orsinolün insan SW480 kolorektal kanser hücrelerinde hücre proliferasyonu ve apoptoz üzerine etkinliğinin araştırılması amaçlanmıştır.*
Gereç ve Yöntem: *Çalışma kapsamında SW480 insan kolorektal kanser hücreleri kullanılmış ve DMEM besiyerinde kültüre edilmiştir. Orsinol, dimetilsülfoksit ile çözülerek stok çözeltisi hazırlanmış ve hücrelere 1-25 mM konsantrasyon aralığında uygulanmıştır. Orsinolün hücre canlılığı üzerine etkisi MTT testi ile belirlenmiştir. Bileşiğin apoptotik etkinliği Annexin V bağlanma analizi ile Muse Hücre Analiz cihazı kullanılarak değerlendirilmiştir.*
Sonuç ve Tartışma: *MTT analiz sonuçları, orsinolün 5 mM ve üzerinde hücre canlılığını doza bağlı olarak anlamlı şekilde azalttığını gösterdi ($p<0.05$). Kontrol grubunda hücre canlılığı 100.00±6.14% iken, 25 mM orsinol uygulanan hücrelerde canlılık 12.50±0.65% olarak belirlendi ($p<0.0001$). Annexin V bağlanma analizi bulgularına göre erken apoptotik hücre popülasyonu 25 mM orsinol uygulanan grupta 12.06±1.22% iken, kontrol grubunda 0.60±0.11% olarak belirlendi. Çalışmadan elde edilen bulgular, orsinol'ün, SW480 kolorektal kanser hücreleri üzerinde yüksek konsantrasyonda sitotoksik etkili olduğunu göstermiş olup, daha ileri çalışmalar ile bileşiğin etkinliğinin artırılmasına ve etki mekanizmasının aydınlatılmasına ihtiyaç duyulmaktadır.*
Anahtar Kelimeler: *Apoptoz, kolorektal kanser, orsinol, proliferasyon, SW480.*

INTRODUCTION

Colorectal cancer (CRC) is a heterogeneous disease that occurs in the gastrointestinal tract, colon and rectum. According to Globocan 2020 data, while the number of new colorectal cancer cases in the world constitutes 10% of all cancers, the mortality rate due to colorectal cancer is 9.4% [1]. It has been reported that the incidence and mortality of colorectal cancer in women is approximately 25% lower than in men [2]. With continued increase in developing countries, the worldwide incidence of colorectal cancer is estimated to rise to 2.5 million new cases by 2035 [2,3]. According to cancer statistics, colorectal cancer ranks third in men and women in Turkey and is seen with a frequency of 23.1 per hundred thousand in men and 14.4 per hundred thousand in women [4]. Although mortality has decreased moderately with the effect of many therapeutic advances worldwide in recent years, the 5-year survival rate in metastatic patients is less than 10% [5].

The development of colorectal cancer is associated with many risk factors, including genetic predisposition, smoking, alcohol, excessive consumption of red meat and processed food, obesity, and decreased physical activity [6,7]. The methods used in the treatment of colon cancer, including the traditional methods of surgery, radiotherapy and current chemotherapy options, are generally low in efficacy and have many side effects. All these problems increase the importance of developing new therapeutically effective compounds in the treatment of colorectal cancer [8].

Natural products and their derivatives have higher efficiency and lower toxicity compared to chemical agents and are less likely to develop multidrug resistance. These products contain many bioactive anticancer compounds and can provide an effective alternative drug therapy for many cancers [9]. Some natural compounds have become productive sources of new anticancer drugs due to their therapeutic advantages, and about 50% of currently used anticancer drugs are directly or indirectly derived from natural products with various structures, including alkaloids, polysaccharides, polyphenols, diterpenoids and unsaturated fatty acids [10].

Orcinol is a natural organic phenolic compound that can be obtained from many lichen species,

including Roccella tinctoria and Lecanora [11]. Studies have revealed that orcinol exhibits antioxidant, antidepressant, adaptogenic and neuroprotective activities [12-15]. The strong radical scavenging activity of orcinol was also demonstrated in Raw 264.7 macrophage cells [16]. In addition, it has been demonstrated that polymer-lipid hybrid nanostructured lipid carrier systems loaded with orcinol glucosides have strong anticancer effects in colon, stomach and liver cancer cell lines, regarding that this system can be developed orally and be preferred in treatment as a good chemotherapeutic agent [17].

The present study aimed to evaluate the effect of orcinol cell viability and apoptosis of SW480 human colorectal cancer cells.

MATERIAL AND METHOD

Cell line and Cell Culture

Human colorectal cancer cell line SW480 (CCL-228) was obtained from the American Type Culture Collection (ATCC, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Biowest, USA) consisted of 10% fetal bovine serum (FBS) (Biowest, USA), 1% L-glutamine (Biowest, USA), and 1% penicillin/streptomycin (Biowest, USA) within a 5% CO2 humidified atmosphere at 37°C.

Orcinol (Sigma, USA) was dissolved in dimethylsulfoxide (DMSO) and the final DMSO concentration was less than 0.1%.

Cell Viability Assay

The effect of orcinol on the viability of SW480 cells was analyzed by MTT assay. The cells were seeded at a density of 9x103cells/well and treated with orcinol at 1, 5, 10, 15, 20 and 25 mM concentrations, and incubated for 24 h. The cells treated with DMSO were used as control. After incubation, the cells were treated with MTT solution (5 mg/mL) and incubated at 37°C for 2 h. The formazan crystals were dissolved in DMSO. The absorbance at 540 nm was determined by a microplate reader (Thermo, Germany). Data were represented as mean ± standard deviation (± SD) from two independent experiments.

Annexin V Binding Assay

SW480 cells were seeded at a density of 2×10^5 cells per well and incubated for 24 hr. The cells were then treated with 1, 5, 10, 20 and 25 mM of orcinol for 24 h. Following incubation, the cells were washed with PBS, and apoptotic cell amount was determined through Annexin V assay kit (Luminex, Germany) by Muse Cell Analyzer (Millipore, Germany).

Statistical Analysis

GraphPad Prism 6.0 version (GraphPad Software Inc.) was used for statistical analysis. The data of cell culture experiments were expressed as mean ±SD and One-way ANOVA test was performed for multiple comparisons.

RESULT AND DISCUSSION

Human colorectal cancer is the leading cause of cancer-related death in almost all developed countries and is the second most common type of cancer worldwide. Half of all patients diagnosed with colorectal cancer die as a result of the disease. Less than 10% of patients with metastatic colorectal cancer can survive for a maximum of five years after diagnosis [18]. Current colorectal cancer treatment is a surgical resection combined with chemotherapy using cytotoxic drugs and radiation therapy. Since the applied treatment is moderately successful for late-stage cancers, it increases the importance of preventive strategies and new approaches in the treatment of colorectal cancer [19,20]. In this context, studies on the effectiveness of natural origin products in cancer treatment are being carried out quite intensively [21,22].

Lichens are complex symbiotic associations consisting of a fungus and an algae. Currently, thousands of primary and secondary metabolites obtained from lichens have been identified [23]. The use of lichens in medicine is mainly based on the content of various biologically active substances with antimicrobial effects. Lichen metabolites show wide biological effects such as antibiotic, antimycotic, antiviral, anti-inflammatory, analgesic, antipyretic, antiproliferative and cytotoxic effects [23-26]. Secondary metabolites of lichens also have strong antioxidant effects. These are substances with a high ability to scavenge toxic free radicals due to their phenolic groups [27].

Orcinol is one of the secondary metabolites of lichens and it has been reported that phenolic orcinol glucosides have potential antioxidant activity, antidepressant activity and immunomodulatory effects [28-30].

At present study, the cytotoxic and apoptotic effects of orcinol in human SW480 colorectal carcinoma cells were experimentally investigated. The cytotoxic effect of the orcinol compound at various concentrations was determined by MTT cell viability assay and the results showed that orcinol significantly decreased the viability of SW480 cells at 5 mM and higher concentrations. The cell viability decreased to 84.84±1.69% at 5 mM ($p<0.05$), while it was determined as 46.94±1.97%, 31.98±1.56%, 13.88±0.63% and 12.50±0.65% at 10, 15, 20 and 25 mM concentrations, respectively ($p<0.0001$) compared to control (Table 1, Figure 1).

Table 1. Viable cell amount % of SW480 colorectal cancer cells.

Concentration (mM)	Viable cell amount (%)	P value (vs control)
0	100.00±6.14	
1	108.79±5.19	0.0217
5	84.84±1.69	<0.0001
10	46.94±1.97	<0.0001
15	31.98±1.56	<0.0001
20	13.88±0.63	<0.0001
25	12.50±0.65	<0.0001

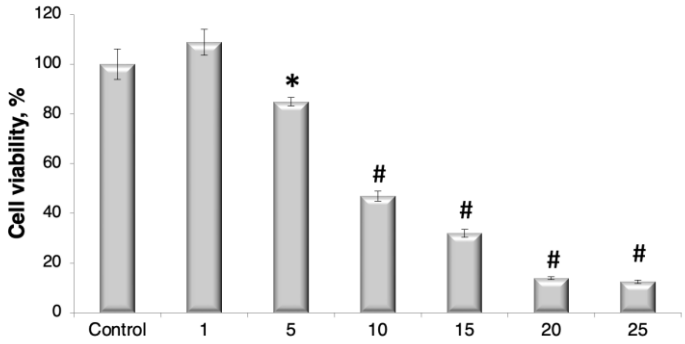

Figure 1. The effect of orcinol on viability of SW480 human colorectal cancer cells. The cell viability was determined by MTT assay. The cells were seeded at a density of 9x103 cell/well and then treated with 1-25 mM orcinol for 24 h. Following incubation MTT dye (5 mg/ml) was added into the wells and the formazan crystals were dissolved in DMSO. The absorbance at 540 nm was recorded. The results are expressed as percentage of live cells compared with untreated control. The data were given as mean±SD of three independent experiments. The differences are * $p<0.05$, # $p<0.0001$ vs control.

The apoptotic effect of orcinol on SW480 colorectal cancer cells were evaluated by Annexin V binding assay. The cell population % values and plot images of flow cytometry analyses were given in Figure 2,3, respectively in which the cell population were defined as percentage of live, early apoptotic (annexin V +), late apoptotic (annexin V +, 7-aminoactinomycine D +), and dead cells. Our results

showed that the early apoptotic cell population % increased to 12.06±1.22% at 25 mM orcinol treatment while it was 0.60±0.11% in control group ($p<0.0001$). The total apoptotic cell population were significantly higher at 5 mM and higher concentration of orcinol when compared to control ($p<0.05$).

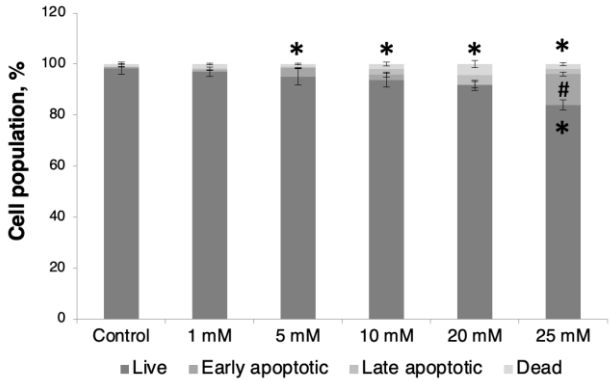

Figure 2. The cell population % of annexin V binding assay. The cells were seeded in 6-well plate and treated with orcinol at 1-25 mM concentrations for 24 h. Four different cell population were determined by Muse cell analyzer including live (annexin V-, 7-AAD-), early apoptotic (annexin V+, 7-AAD-), late apoptotic (annexin V+, 7-AAD+), dead (7-AAD+). Data are expressed as mean±SD (n=3) of cell population %. Significant differences are * * $p<0.05$, # $p<0.0001$ vs control.

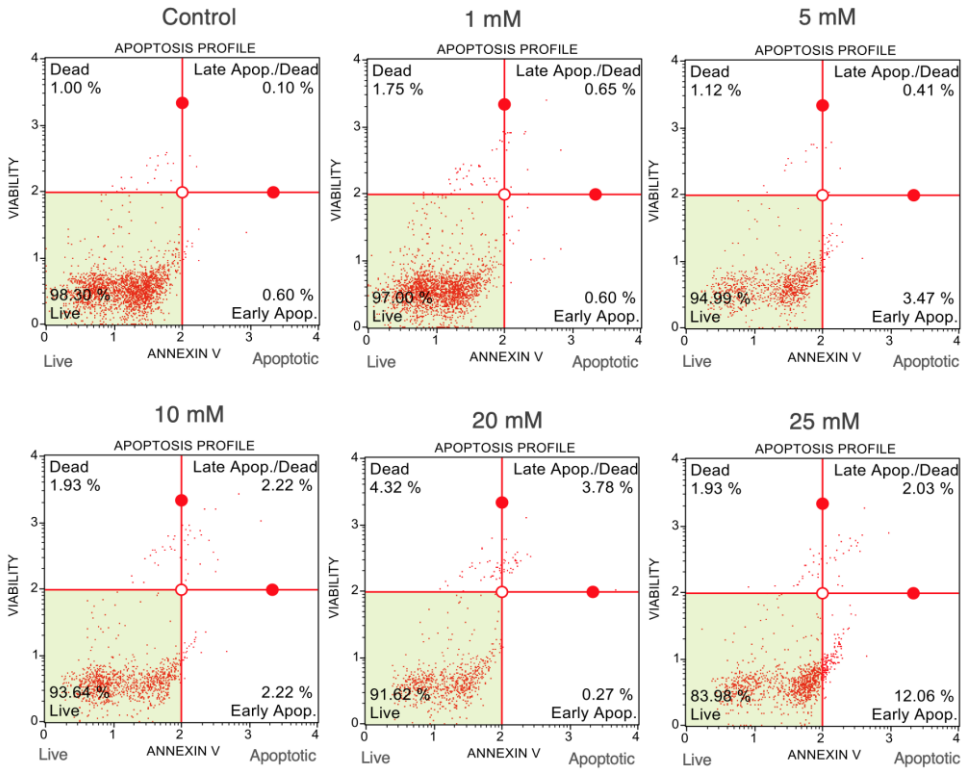

Figure 3. The plot graphs of annexin V binding assay. The cells were treated with 1-25 mM of orcinol and Muse cell analyzer (Merck Millipore) was used to determine the apoptotic cell population. Based on the Annexin V and 7-AAD positivity, the apoptotic and dead cell population % were determined, respectively. The live, early apoptotic, late apoptotic and dead cell population were detected through cytofluorometric separation on cell analyzer. The figure represents dot plots of three independent experiments.

There are various studies in the literature on the efficacy of orcinol in cancer cells. In a study, the potential cytotoxic activities of different compounds derived from phloroglucinol and orcinol in PC3 and DU145 prostate cancer cell lines, MDA-MB231 breast cancer cells, HT29 colon cancer cells and human dermal fibroblasts, a non-tumoral cell line, were evaluated. It has been determined that the cytotoxic activity of compounds derived from orcinol against cells and their effects on chromatin core condensation and/or fragmentation induction, mitochondrial membrane potential and caspase-3 activity are variable [31].

In another study investigating the immunomodulatory activity of thirteen lichen metabolites, including orcinol, orsellinic acid, and methyl orsellinate, it was determined that all of these metabolites had antioxidant activity, however, the immunomodulatory activity of orcinol was low [32]. A study evaluating the cytotoxicity of 5-n-alkylresorcinol homologues and its fraction on mouse fibroblast cell line L929, it was reported that orcinol showed low cytotoxicity among isolated compounds [33].

In conclusion, our study determined that orcinol had a dose-dependent cytotoxicity and apoptotic activity in SW480 human colorectal cancer cells. Further studies are required to increase the efficiency of the compound in question and to elucidate its mechanism of action.

AUTHOR CONTRIBUTIONS

Concept: F.B.A.; Design: F.B.A.; Control: F.B.A.; Sources: F.B.A.; Materials: B.Y., F.B.A.; Analysis and/or Interpretation: B.Y., F.B.A.; Literature Review: B.Y., F.B.A.; Manuscript Writing: B.Y., F.B.A.; Critical Review: F.B.A.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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