JBACHS
Journal of Basic and Clinical Health Sciences

J Basic Clin Health Sci 2019; 3:78-82
https://doi.org/10.30621/jbachs.2019.610

Original investigation

Anticancer Efficiency of Curcumin on Ovarian Cancer

Gizem Calibasi Kocal[1]

[1]Dokuz Eylul University, Institute of Oncology, Department of Translational Oncology, Izmir, Turkey

Address for Correspondence: Gizem Calibasi Kocal, **E-mail:** gizemcalibasi@gmail.com
Received: 08.04.2019; **Accepted:** 11.04.2019; **Available Online Date:** 28.05.2019

Cite this article as: Calibasi Kocal G. Anticancer Efficiency of Curcumin on Ovarian Cancer. J Basic Clin Health Sci 2019; 3:78-82. https://doi.org/10.30621/jbachs.2019.610

Abstract

Objectives: Ovarian cancer is one of the most common malignancies in women, and it is quite difficult to cure. In order to treat the disease, chemotherapy is often performed as adjuvant therapy following the surgery or neo-adjuvant cure for the pre-operative purpose. While the strategy is an effective way to destroy the tumor, it also affects healthy cells thus it causes side effects leading to even multiple organ failure and death. So that to minimize the disadvantage of chemotherapeutics, phytochemicals have been studied as chemotherapeutic agents. Curcumin is an orange-yellow nutraceutical, which is isolated from turmeric (Curcuma longa L.). This phyto-polyphenolic compound has various roles in bio-industry such as textile and food dye as well as a wide range of medicinal properties especially the anticancer feature.

Methods: The aim of this study is to investigate the anti-proliferative and anti-migrative effects of curcumin on ovarian adenocarcinoma. Cell proliferation rates on ONCO-DG-1 cell line were examined in five different doses of curcumin. Wound healing assay was performed to evaluate the anti-migrative effect on curcumin on this cell line.

Results: The results showed that curcumin inhibits tumor cell proliferation at 5, 10, 20, 30 and 40 μM doses at 24- and 48-hours incubation time ($p<0.05$). IC50 dose of curcumin is determined as 17 μM on ONCO-DG-1 cell line. Wound healing assays showed that curcumin inhibits the wound closure with its anti-migrative effect on ONCO-DG-1 cell line.

Conclusion: Due to the anticancer features of curcumin, it can be considered as a promising chemotherapeutic agent in the treatment of ovarian adenocarcinoma.

Keywords: Curcumin, Ovarian Cancer, Anticancer, Antiproliferative, Antimigrative, ONCO-DG-1

INTRODUCTION

The diagnosis of ovarian cancer is the beginning of a long and difficult journey for a patient. The treatment of the disease causes side effects on the half of the patients who are under treatment. Accepted and common chemotherapeutic agents such as cisplatin, carboplatin, paclitaxel and docetaxel are toxic for both cancerous and normal cells, therefore they cause debilitating side effects and limit the treatment. Moreover, most ovarian cancer patients will eventually recur, and die of the disease (1). Therefore, identification of new promising chemotherapeutic drug with low adverse effects is important. Phytochemicals are drawn interest since they are considered to have no harmful effects on healthy cells while neutralizing the malignancy.

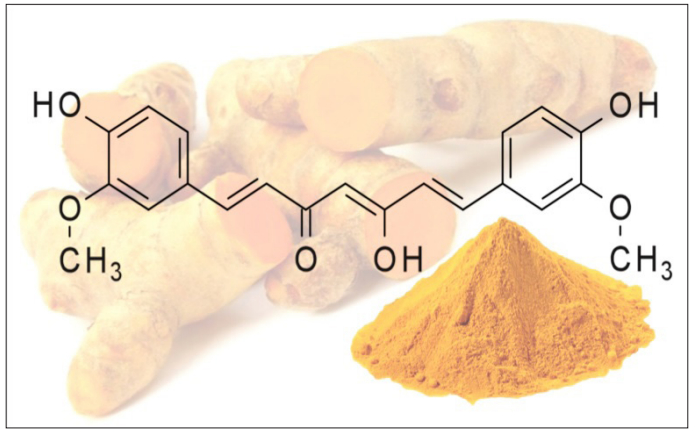

Figure 1. Chemical structure of curcumin.

Curcumin, [1,7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], is a natural orange-yellow phenolic compound which extracted from the natural plant turmeric (*Curcuma longa* L.) (Figure 1). Curcumin, the most abundant metabolite in turmer-

ic, is a multi-functional and pharmacologically safe natural agent. Other metabolites in the plant are demethoxycurcumin and bis-demethoxycurcumin (2–4).

In the last decades, wide-ranging potential therapeutic actions of curcumin, including antioxidant, anti-inflammatory, anti-infectious, anti-fibrotic, and cancer-preventive, in both cell and animal disease models have been investigated (5, 6).

The inhibitory and down-regulatory effects of curcumin on tumor biology-associated molecules, such as nuclear factor-kappa B (NF-κB), Akt, cyclooxygenase-2 (COX-2), 5-lipooxygenase, vascular endothelial growth factor (VEGF), phosphorylated signal transducers and activators of transcription 3 (STAT3) and matrix metalloproteinase-9 (MMP-9), were also demonstrated in different studies (7, 8). This is important because the activation or aberrant expression levels of significant molecules in tumor cells, are responsible for the development of carcinogenesis, such as antiapoptotic genes, metastasis, tumor promotion, and malignancy. Therefore, anticancer property of curcumin has been demonstrated on lung, breast, colorectal and prostate cancer-based studies (1, 9). The aim of this study is to investigate the anticancer effects of curcumin on the ovarian adenocarcinoma cells, ONCO-DG-1.

MATERIALS and METHOD

Cell culture

Human ovarian adenocarcinoma ONCO-DG-1 (*DSMZ no: ACC507*) cell line was obtained from DSMZ (*Leibniz Institute DSMZ-German Microorganism and Cell Culture Collection Braunschweig, Germany*) and cultured in RPMI 1640 medium (*Biochrom GbmH, Berlin, Germany*) containing 10% fetal bovine serum (*FBS, Cegrogen Biotech GmbH, Stadtallendorf, Germany*) and 1% penicillin/streptomycin (*Biochrom GbmH, Berlin, Germany*). Cells were incubated in 5% CO_2 incubator at 37°C in humidified air.

Preparation of curcumin stock solution

Curcumin (*Sigma-Aldrich, St Louis, MO, USA*) was reconstituted in dimethyl sulfoxide (DMSO) to obtain stock solutions for further dilutions. Stocks were stored at –20°C and kept light protected. Different concentrations (5, 10, 20, 30 and 40 µM) of curcumin were prepared by diluting the stock solution with DMSO.

Cell proliferation and viability

Real-time analysis of cell proliferation and viability was performed by using the xCELLigence RTCA (Real-Time Cell Analyzer) SP (Single Plate) instrument (*ACEA Biosciences, Inc. San Diego, USA*). To monitor the viability of cultured cells, the RTCA instrument measures the electrical impedance as the readout. As the whole instrument is placed inside of an incubator, the cells are kept at optimal culture conditions (5% CO_2, 37°C) during the proliferation/viability assay, which was performed according to the manufacturer instructions. ONCO-DG-1 cells were seeded in the disposable E-plate 96, a microtiter plate with gold electrodes at the bottom of each well, separately at a density of 1x 10^4 cells/well. After 24 hours of incubation, five different curcumin concentrations, as 5, 10, 20, 30 and 40 µM, were applied to the cells. Samples free of the curcumin added cell culture media served as control samples. After curcumin application, the cells were cultured for 48 hours. Each concentration was studied triplicate. In order to detect the cell proliferation rate, electrical impedance changes were measured per 15 minutes through the gold microelectrode on the bottom of the plate.

Wound healing assay

ONCO-DG-1 cells were seeded on a 24-well plate (20 x 10^4 cells/well). After confluency of the cells, wells were scratched with the 20 µl pipette tip. The 1% FBS-supplemented complete medium was used as a negative, and 20% FBS-supplemented medium was used as a positive control. IC50 dose of curcumin was added to 1% and 20% FBS-supplemented media, individually. Inverted microscope (*Carl Zeiss Suzhou Co., Ltd, Axio Ver. A1*) was used to monitor wound healing. ImageJ software 1.49 was used to measure the wounds. The test was performed in triplicate.

Statistical analysis

Mann-Whitney U Analysis was used to determine the significance between curcumin and its activity. SPSS (*Version 15.0; SPSS, Inc., Chicago, IL, USA*) was used for all analysis. The level of statistical significance was set at $p<0.05$. All tests were performed in triplicate.

RESULTS

Proliferation/viability assays performed on ONCO-DG-1 cell line, was determined in a dose dependent manner by using xCelligence RTCA SP method. The impedance measurement during the proliferation/viability assay is displayed as a cell index (CI), which provides quantitative information regarding the biological status of the cells, including cell number and viability (Figure 2A). The results of this experiment showed that curcumin inhibits tumor cell proliferation on all applied doses at 24-and 48-hours incubation time ($p<0.05$) (Figure 2B and C). When the viability results of same curcumin dose compared between 24 hours and 48 hours, significant differences ($p<0.05$) were observed on cells exposed to 20, 30 and 40 µM curcumin doses (Figure 2D). IC50 dose of curcumin on the ONCO-DG-1 cell line was found as 17 µM.

Migration rate of ovarian cancer cell line was tested via wound healing assay and percentage of wound closure was calculated by Image J. software. IC50 dose of curcumin was added to 1% and 20% FBS-supplemented cell culture media. After 48 h treatment, the wound closures were recorded. Wound closure percentages of negative control group which includes 1% FBS-supplemented culture media, curcumin added 1% FBS-supplemented culture media group, positive control group which includes 20% FBS-supplemented culture media, and curcumin added% 20 FBS-supplemented culture media group were exhibited as 6.6%,-8.3%, 19.6% and-5.2%, respectively (Figure 3). Interestingly, negative percentages represent an expanded gap. When the wound closure rates compared between each curcumin added condition and its own control group, curcumin shows significant effect on closure of the wounds ($p<0.05$). These results indicate that curcumin inhibits the wound healing with its anti-migrative effect on ONCO-DG-1 cell line.

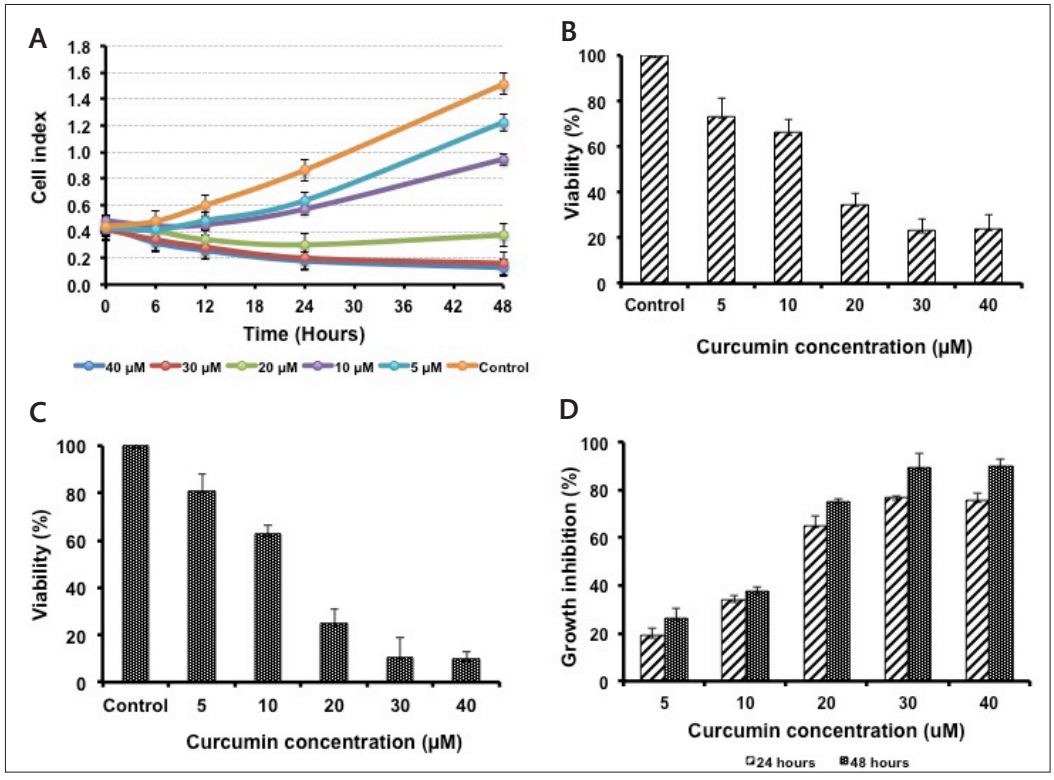

Figure 2. Proliferation/viability results of curcumin. **A)** Time-cell index graph showing the cytotoxic effect of curcumin at 5 different doses (5, 10, 20, 30, 40µM) applied on ONCO-DG-1 cell line. All viability analysis was performed as triplicate. **B)** Viability (%) results of curcumin applied cells after 24-hour incubation. **C)** Viability (%) results of curcumin applied cells after 48-hour incubation. **D)** Growth inhibition rate (%) of curcumin at 5 different doses after 24-hour and 48-hour incubation.

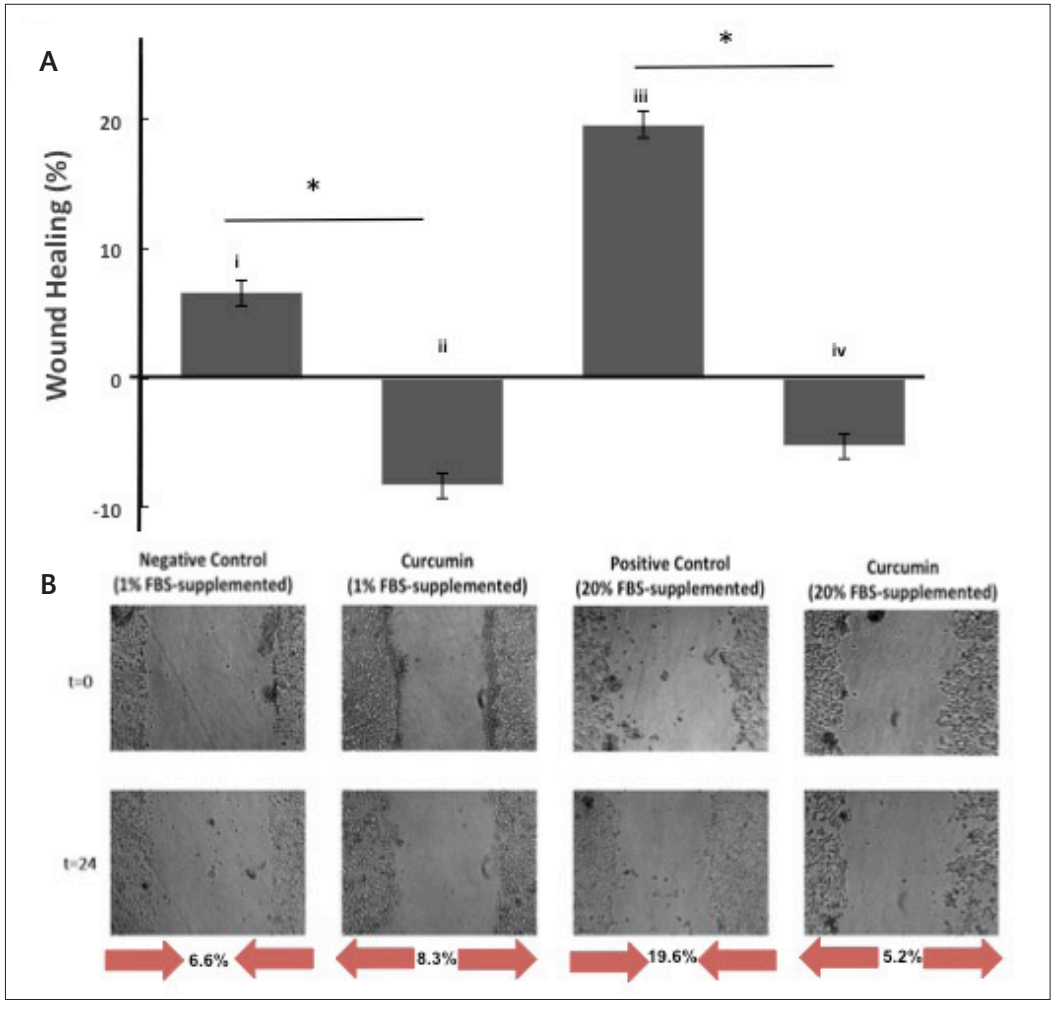

Figure 3. Results of wound healing assay on ONCO-DG-1 cell line after curcumin application. **A)** Bar graphs of wound healing assay after 24 hours of incubation i) 6.6% wound closure for negative control group which includes 1% FBS-supplemented culture media; (ii) -8.3% wound closure for 1% FBS-supplemented culture media with curcumin; (iii) 19.6% wound closure for positive control group which includes 20% FBS-supplemented culture media; (iv) -5.2% wound closure for %20 FBS-supplemented culture media with curcumin. **B)** Wound healing pictures of ONCO-DG-1 cell line at 0th and 24th hours.

DISCUSSION

Chemotherapy related adverse side effects and drug resistance, which is developed during treatment, are the reasons to explore into new anti-cancer drugs. Medicinal plants and natural phytochemicals isolated from plants have been used as traditional treatment options for various diseases for many years in different parts of the world, so they are considered as good promising source. Turmeric (*Curcuma longa* L.) is a flavorful yellow-orange spice, and curcumin is its main component (10).

In this study, the cytotoxic and anti-migrative properties of curcumin on ovarian adenocarcinoma were investigated. There was a significant difference on viability results for ONCO-DG-1 cells treated with curcumin in a dose-dependent manner. After 48 hours of curcumin treatments, ONCO-DG-1 ovarian adenocarcinoma cell densities decreased dramatically compared to control samples, which includes cell medium only. Also, the data point out that an anti-migration effect of curcumin via wound healing assay. So these results suggest that curcumin represent a promising agent feature on ovarian cancer. There was a significant difference on wound closure percentages between each curcumin added condition and its own control group ($p<0.05$). However, there is a need for sufficient evidence to illuminate the path of curcumin effect on ONCO-DG-1 cell, as well as ovarian adenocarcinoma.

The inhibitory and down-regulatory effects of curcumin on tumor biology-associated molecules, such as nuclear factor-kappa B (NF-κB), Akt, cyclooxygenase-2 (COX-2), 5-lipooxygenase, vascular endothelial growth factor (VEGF), phosphorylated signal transducers and activators of transcription 3 (STAT3) and matrix metalloproteinase-9 (MMP-9), were demonstrated in different studies (7, 8). This is important because the activation or aberrant expression levels of significant molecules in tumor cells, are responsible for the development of carcinogenesis, such as antiapoptotic genes, metastasis, tumor promotion, and malignancy. Previous studies have also highlighted, antiproliferative feature of curcumin on breast cancer, colorectal cancer, and osteosarcoma cell lines (11–13).

Syng-ai C, et al, focused the cytotoxic effects of various concentration of curcumin (10, 25, 50, 100 μM) on breast cancer and hepatocellular carcinoma cell lines. It was shown that curcumin inhibited proliferation in MCF-7, MDAMB, and HepG2 cells in a dose-dependent and time-dependent manner via inducing apoptosis. In our study, we also observed the similar proliferation inhibitory effect of curcumin in 10 and 20 μM curcumin concentrations (14).

In another study, Caco-2 colorectal cancer cells were exposed to curcumin at concentrations ranging from 5 to 100 μM (5, 10, 25, 50 and 100 μM) for 24 hours, and a significant suppression was reported even at a 5 μM-curcumin. In our study, higher dose of curcumin was not applied, but we also observed significant growth inhibition even at 5 μM-curcumin at 24 hours. In the same study by Sakuma S, a dose dependent inhibition in cell growth was also determined, and apoptosis was induced through an increase in the Bax/Bcl-2 ratio and activation of caspase-3/7 (12).

As a result of cytotoxicity test on ovarian cancer with curcumin, *Mingxin Shi et al.* (15) found the IC50 dose of curcumin on Ho-8910 cell line as 40 μM. In our study, IC50 dose of curcumin on ONCO-DG-1 cell line was found as 17 μM and the phytochemical showed an cytotoxic effect on this cell line between 5–40 μM. Ho-8910 cell line was established from the ascitic fluid of a patient with poorly-differentiated ovarian papillary serous cyst-adenocarcinoma. Due to the highly metastatic feature of Ho-8910 cell line, IC50 value can be higher from our results. *Lin G, et al*, investigated the effect of curcumin on metastatic human ovarian cancer cell lines, SKOV3ip1 and HeyA8 (16). According to the results they observed cytotoxic effect of curcumin on different concentrations as 1, 5, 10, 25 and 50 μM. Results from our study were also supported their results. In the same study, the effect of curcumin on apoptosis was also found in HeyA8 and SKOV3ip1 cells.

Based on significant efficacy in preclinical models, curcumin-based therapies may be attractive in the treatment of ovarian carcinoma. In the light of this study, the effect of curcumin on cell proliferation in ovarian cancer needs to be investigated in terms of proliferation related pathways. Moreover, studies on drug combinations should be also carried out to effective dose of curcumin and related pathways into the diseased tissue.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - GCK; Design - GCK; Supervision - GCK; Fundings - GCK; Materials - GCK; Data Collection and/or Processing - GCK; Analysis and/or Interpretation - GCK; Literature Search - GCK; Writing Manuscript - GCK; Critical Review - GCK

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study has received no financial support.

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