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# Cytotoxic/Proliferative Effects of Umbelliprenin on Colon Cancer Cell Lines

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**Background:** Colon carcinoma growth depends on many factors such as, different organisms, and immune cells, which induce and produce inflammatory cytokines. Umbelliprenin is a naturally prenylated coumarin with anti-inflammatory activities. Its ability to induce cancer cell death has shown variation on different cancer cell lines.
**Objectives:** 7-Prenyloxycoumarins, including umbelliprenin have been widely investigated because of their pharmacological activities. This paper shows the effect of umbelliprenin on colon cancer cell lines.
**Material and Methods:** In the present study, invasive SW48 and noninvasive SW1116 were treated with umbelliprenin (6.25, 12.5, 25, 50, 100, and 200 μM), and the cytotoxicity was determined using a methyl thiazolelydiphenyl-tetrazolium bromide (MTT) assay.
**Results:** Umbelliprenin had significant cytotoxic activity against SW48 cells at all study concentrations (except for 6.25 μM), with IC50 values of 117, 77, and 69 μM after 24, 48, and 72 h, respectively. However, it was cytotoxic against SW1116 only at higher concentrations of 100 and 200 μM (34% and 64% cell death). At lower concentrations, umbelliprenin showed a significant proliferative effect on this noninvasive cancer cell line. Our data were validated by eye and microscopic images.
**Conclusions:** We found a moderate cytotoxic activity of umbelliprenin against invasive SW48 cells, and both cytotoxic and proliferative effects on noninvasive SW1116 cells. Therefore, using umbelliprenin as an anti-inflammatory or cytotoxic compound for patients with colon cancer should be used with care.

*Keywords:* Apoptosis; Colorectal Neoplasms; Umbelliprenin

## 1. Background

Colon carcinoma is among the most frequent cause of cancer deaths in the majority of countries (1, 2). The disease occurs in the presence of gut flora and numerous myeloid and lymphoid cells, which induce and produce pro-inflammatory cytokines (3). Emerging evidence has demonstrated the contribution of chronic inflammation to neoplastic transformation by a variety of mechanisms (4). Treatment regimens for patients with colon carcinoma including surgery, chemotherapy, and molecular targeted therapy, have been improved. Furthermore, finding novel therapeutic targets or some supplements with anti-inflammatory and/or apoptotic properties is still under intensive research (5, 6).

7-Prenyloxycoumarins, a group of secondary metabolites, are found in plants belonging to the families of Rutaceae and Umbelliferae. Three of these metabolites; auraptene, umbelliprenin, and 7-isopentenyloxycoumarin, have recently gained attention from researchers due to their biological and pharmacological activities (7). Umbelliprenin is synthesized by various Ferula species, and it is a constituent of a number of plant species consumed as food such as; celery, Angelica archangelica, Coriandrum sativum, and Citrus limon (7).

Umbelliprenin can exert antitumor activity via four main pathways. Firstly, it has been shown that umbelliprenin has a remarkable matrix metalloproteinase (MMP) inhibitory effect, interestingly, at minimal toxic dose levels (8). MMPs play critical roles in tumor invasion and the inflammatory process (8). Secondly, in vitro and in vivo models have provided evidence of antilipoxygenase, antioxidants; and anti-inflammatory properties for umbelliprenin (9, 10). Chronic inflammation and free radicals can accelerate several aspects of tumorigenesis (9, 11). Thirdly, this compound was proven to be chemopreventive, as it delayed the formation of papilloma in a mouse model (12). Lastly, it has the ability to induce apoptosis in cancer cell lines. It was particularly cytotoxic against M4Beu metastatic pigmentated malignant melanoma cells, with cell-cycle arrest in G1, and induction of caspase-dependent apoptosis (13). However, the ability of umbelliprenin to induce apoptosis

**Implication for health policy/practice/research/medical education:**
Umbelliprenin might have proliferative effects on colon cancer; therefore, its possible administration for patients with colorectal cancer should be with caution and care.

varies in different cancer cell lines (12-14). The proliferative effects of umbelliprenin on normal immune cells have been reported to be insignificant (14). No significant proliferative activity of this compound has been reported on cancer cells, and a limited number of publications are available regarding umbelliprenin and colon cancer (13, 15). No significant effect of umbelliprenin on the proliferation of DLD1 cells (invasive human colon adenocarcinoma cell line) have been reported using a resazurin reduction test (RRT) (13). Jabrane et al. showed no significant cytotoxic activity of umbelliprenin against two colorectal cancer cell lines; HT116 (invasive human colon carcinoma), and HT-29 (invasive human Caucasian colon adenocarcinoma cell line) by using a methyl thiazolelydiphenyl-tetrazolium bromide (MTT) assay (15). However, umbelliprenin may differently act on the different cell lines of a particular type of cancer (14). Moreover, colon cancer develops in a potentially inflammatory environment (3), and umbelliprenin might still be useful in colon cancer due to its anti-inflammatory properties (9, 10). Therefore, its effects on colon cancer cell death and proliferation need to be clarified.

## 2. Objectives

In this study we employed a MTT assay, in which a decrease in MTT reduction can occur as a result of cell death or cell proliferation inhibition, to probe the outcomes of umbelliprenin treatment on colon cancer cells. The SW48, an invasive human colon adenocarcinoma cell line, and SW1116, a noninvasive cell line, were treated with umbelliprenin. The IC50 was determined as the half inhibitory concentration of umbelliprenin that led to a 50% decrease in the OD of the test compound compared to the control. Subsequently, trypan blue exclusion dye was used to calculate the percentage of cell death at the $IC_{50}$ value of umbelliprenin.

## 3. Materials and Methods

### 3.1. Cell Culture

SW48 and SW1116 cell lines were purchased from the Pasteur Institute of Iran, and maintained in a complete media containing RPMI-1640 supplemented with 10% (v/v) heat inactivated fetal bovine serum (FBS) (Gibco/BRL, Germany), 100 units/ml penicillin, and 100 μg/ml streptomycin (Biosera, The UK) under standard culture conditions (37˚C, 5% $CO_2$, and 95% humidity). The culture media were changed every 2-3 days.

### 3.2. Umbelliprenin Preparation

Umbelliprenin ($C_{24}H_{30}O_3$, MW: 366) was chemically prepared according to the method described previously by Askari et al. (7). Umbelliprenin was dissolved in 100% dimethyl sulfoxide (DMSO) to 20 μM concentration. The stock solutions were aliquoted and kept in a refrigerator (2-8˚C). Just before the experiment, the stock was diluted in CM10 media at 37˚C to obtain a maximum DMSO concentration of 1% (v/v).

### 3.3. MTT Assay

Exponentially growing SW48 and SW1116 cells were trypsinized, rinsed with phosphate-buffered saline (PBS), seeded onto 96 well plates ($5\times10^3$ cells/well), and allowed 24 hours to become attached. They were then treated with fresh medium containing umbelliprenin at different concentrations (6.25, 12.5, 25, 50, 100, 200 Mm). Untreated cells were used as negative controls and different concentrations of DMSO were used as the solvent control. The plates were incubated for 24, 48, and 72 hours, respectively. Then 10 μl MTT (sigma) was added to each well, and the cells were incubated for 4 hours (37˚C 5% $CO_2$). After discarding the media containing MTT, 150 μL DMSO was added to each well to dissolve the formazan crystals, the plate was foil wrapped and gently mixed at room temperature for 20 min. Finally the optical density values (OD) of the solubilized formazan product were determined by an ELIAS reader (wavelength of 570 nm). Three independent experiments in triplicate were performed for each experiment.

### 3.4. Trypan Blue Staining

At the end of treatment with umbelliprenin, a freshly prepared solution of 10 μM trypan blue (0.05% in distilled water) was mixed to form a cellular suspension, and the number of viable cells (not stained) were counted using a haemocytometer. Nonviable cells appeared blue when stained. The percentage of viability was expressed as the number of cells, excluding those dyed. At least 200 cells were counted per treatment.

### 3.5. Statistical Analysis

Results were evaluated as the mean ± standard deviation (SD) from the three independent experiments. The difference of umbelliprenin treated cells compared to the control cells was measured using a one-way ANOVA analysis, followed by Tukey Post Hoc multiple comparisons using GraphPad Prism (version 5) software. IC50 values were calculated using Curve Expert software version 1.3. The inhibition index (%) was calculated according to the following formula:

$$100-\left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of corresponding control}}\right)\times100$$

Proliferation index (%) was calculated based on the following formula:

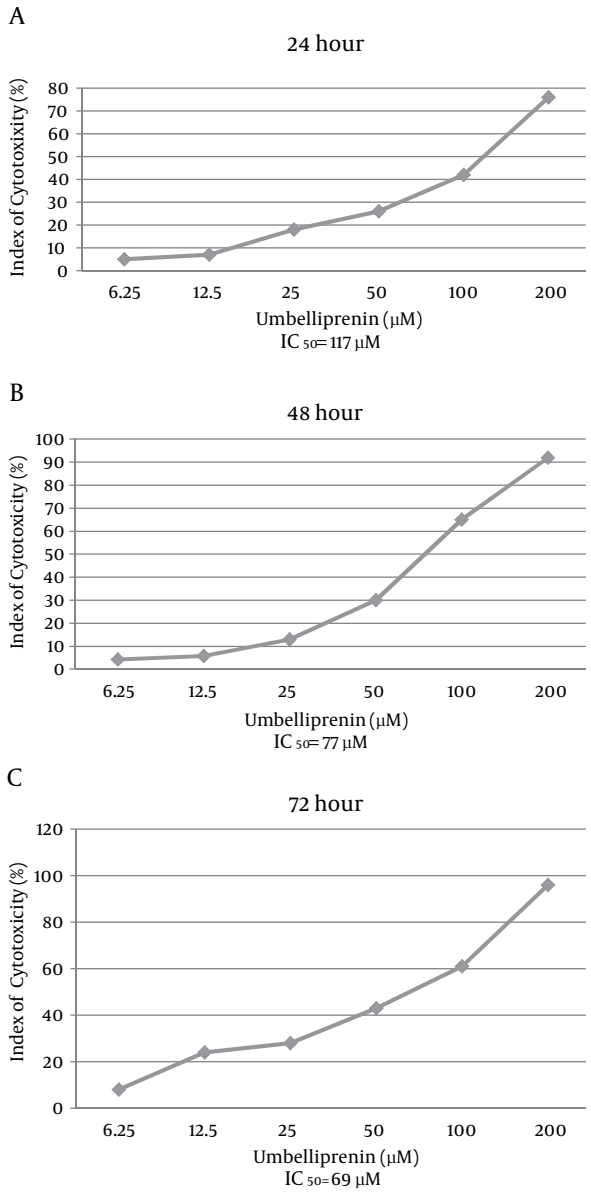
$$\left(\frac{\text{Absorbance of treated cells}}{\text{Absorbance of corresponding control}}\right)\times100$$

$P < 0.05$ was considered significant.

## 4. Results

SW48 and SW1116 cell lines were incubated with various concentrations of umbelliprenin for; 24, 48, and 72 hours, subsequently assayed for apoptosis using a MTT assay. We observed that umbelliprenin was cytotoxic against SW48 cells, and that activity was dose and time-dependent. The IC50 values were found to be 117, 77, and 69 μM after 24, 48, and 72 hours, respectively (Figure 1). At the calculated IC50 value, the cells were treated with umbelliprenin or DMSO. The percentage of cell death was found to be around 50% using trypan blue exclusion dye.

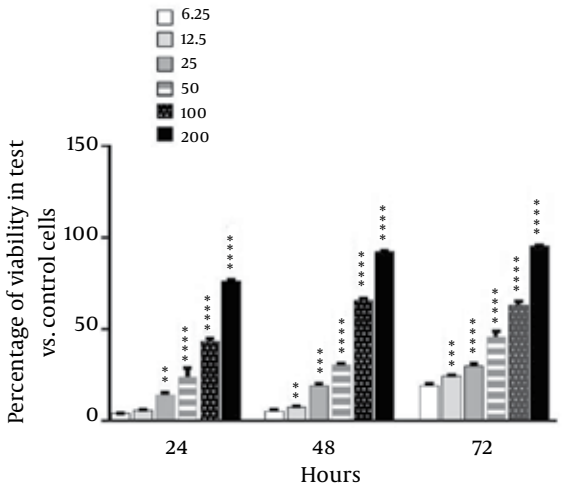
**Figure 1.** Umbelliprenin Induced Apoptosis in a Dose- and Time-Dependent Manner




IC50, 24 hours = 117 μM. IC50, 48 hours = 77 μM. IC50, 72 hours = 69 μM.

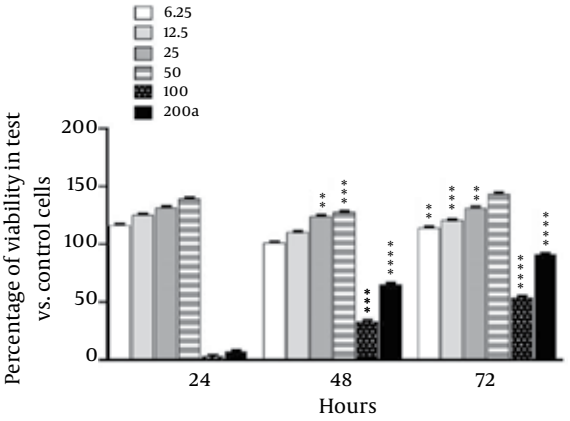
Each concentration of umbelliprenin was compared with the corresponding concentration of DMSO using a one way ANOVA. The three P values for the; 24, 48 and 72hours experiments were all $\leq 0.0001$. As indicated in Figure 2, a Tukey Post Hoc multiple comparisons test revealed that umbelliprenin caused a significant cell death compared to the controls, at concentrations $\geq 25$ μM, in all of the experiments. At a concentration of 12.5 μM, umbelliprenin was cytotoxic after 48 and 72 hours, and it did not cause significant cell death at 6.25 μM concentration in any of the experiments.

**Figure 2.** Effect of Umbelliprenin on SW48 Cells Death Measured by MTT Assay After 24, 48, and 72 hours




Results were expressed as the mean of inhibition index (%) mean (SD); **P < 0.01, ***P < 0.001, ****P < 0.0001; P values represent the differences between umbelliprenin treated and DMSO treated cells.

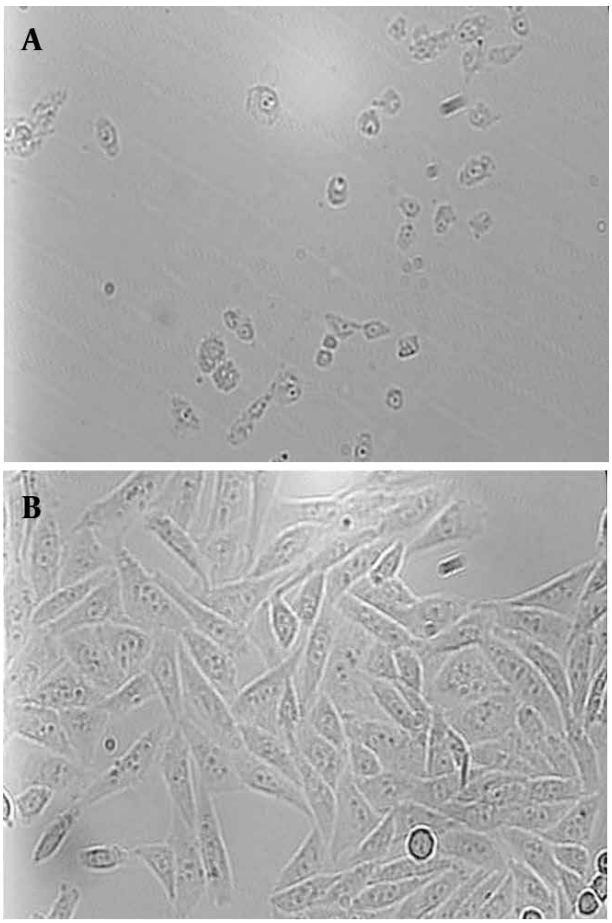
**Figure 3.** Effect of Umbelliprenin on the Viability of SW1116 Cells Measured by MTT Assay After 24, 48, and 72 hours.




Results were expressed as the mean (SD); Stars indicate the significant difference between umbelliprenin treated cells and controls (DMSO treated cells); **P < 0.01, ***P < 0.001, ****P < 0.0001.

The effect of umbelliprenin on SW1116 cells was different. At higher concentrations, 100 and 200 μM of umbelliprenin were cytotoxic and at lower concentrations, 25 and 50 μM showed proliferative effects (Figure 3). These data were clearly consistent with what was observed by microscope and presented in Figure 4.

**Figure 4.** The Effect of Umbelliprenin on SW1116 Cells After 48 Hours.




a) Umbelliprenin induced cell death at 100 and 200μM concentrations. b) Induced proliferation at 50μM concentration.

## 5. Discussion

Umbelliprenin has been shown to be a promising anti-inflammatory and anti-oxidant agent in both in vivo and in vitro models (9, 10). The induction of apoptosis and chemopreventive activities has also been reported for this compound (12-16). However, its apoptotic activity varies in different cell lines (13). Colon cancer occurs in the presence of many organisms and immune cells, and these are potent inducer producers of inflammatory cytokines (3). A limited number of researches have been published regarding umbelliprenin and colorectal cancer with conflicting results (13, 15). Our study was conducted to study the possible cytotoxic effects of umbelliprenin on invasive SW48 and noninvasive SW1116 colon cancer cell lines, the as these two cell lines have no available data concerning umbelliprenin treatment. Umbelliprenin had moderate cytotoxicity against SW48 cells with an IC50 of 77 μM after 48h. It was cytotoxic against SW1116 only at higher concentrations, 100 and 200 μM (34% and 64% cell death), but at lower concentrations, umbelliprenin showed a significant proliferative effect on this noninvasive cancer cell line, which was validated by microscopic images.

Another member of prenylated coumarins, auraptene, has a very similar structure to umbelliprenin. The only difference is the higher length of prenyl moiety in the umbelliprenin structure. Auraptene, with more potent cytotoxicity has been compared to umbelliprenin (10). One can argue that the higher length prenyl moiety in umbelliprenin interferes with its cytotoxic activity, and even reverses this activity. On the other hand, the proliferative effect of umbelliprenin on SW1116 may be its exclusive feature on this cell line model, reflecting the importance of personalized medicine and approach in cancer (17).

In conclusion, we have studied the cytotoxic activity of umbelliprenin against two well-known colon cancer cell models, one invasive, and the other noninvasive. Umbelliprenin was moderately cytotoxic against them, except at lower dose, where it caused the proliferation of noninvasive SW1116 cells. The administration of umbelliprenin either as an anti-inflammatory or cytotoxic compound may increase tumor growth.

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## Authors' Contribution

Z. Mojtahedi, M. Ramezani, A. Ghaderi: Design, data analysis, revising the manuscript draft, and final approval of the manuscript.

M. Hamidinia: Performed the experiment and data acquisition and also prepared the manuscript draft.

## Financial Disclosure

The authors declare no conflict of interest.

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## References

1. Weitz J, Koch M, Debus J, Höhler T, Galle PR, Büchler MW. Colorectal cancer. *Lancet*. 2005;**365**(9454):153–65.
2. Masoompour SM, Yarmohammadi H, Rezaianzadeh A, Lankarani KB. Cancer incidence in southern Iran, 1998-2002: results of population-based cancer registry. *Cancer Epidemiol*. 2011;**35**(5):e42–7.
3. Whiteside TL. What are regulatory T cells (Treg) regulating in

cancer and why? *Semin Cancer Biol.* 2012;**22**(4):327–34.
4. Itzkowitz SH, Yio X. Inflammation and cancer IV. Colorectal cancer in inflammatory bowel disease: the role of inflammation. *Am J Physiol Gastrointest Liver Physiol.* 2004;**287**(1):G7–17.
5. Kantor ED, Lampe JW, Peters U, Shen DD, Vaughan TL, White E. Use of glucosamine and chondroitin supplements and risk of colorectal cancer. *Cancer Causes Control.* 2013;**24**(6):1137–46.
6. Villasenor A, Ballard-Barbash R, Ambs A, Bernstein L, Baumgartner K, Baumgartner R, et al. Associations of serum 25-hydroxyvitamin D with overall and breast cancer-specific mortality in a multiethnic cohort of breast cancer survivors. *Cancer Causes Control.* 2013;**24**(4):759–67.
7. Askari M, SAHEBKAR AH, Iranshahi M. Synthesis and purification of 7-prenyloxycoumarins and herniarin as bioactive natural coumarins. *Iran J Basic Med Sci.* 2009;**12**(2):63–9.
8. Shahverdi AR, Saadat F, Khorramizadeh MR, Iranshahi M, Khoshayand MR. Two matrix metalloproteinases inhibitors from Ferula persica var. persica. *Phytomedicine.* 2006;**13**(9-10):712–7.
9. Iranshahi M, Askari M, Sahebkar A, Adjipavlou-Litina D. Evaluation of antioxidant, anti-inflammatory and lipoxygenase inhibitory activities of the prenylated coumarin umbelliprenin. *DARU.* 2009;**17**(2):99–103.
10. Soltani F, Mosaffa F, Iranshahi M, Karimi G, Malekaneh M, Haghighi F, et al. Evaluation of antigenotoxicity effects of umbelliprenin on human peripheral lymphocytes exposed to oxidative stress. *Cell Biol Toxicol.* 2009;**25**(3):291–6.
11. Cheon EC, Khazaie K, Khan MW, Strouch MJ, Krantz SB, Phillips J, et al. Mast cell 5-lipoxygenase activity promotes intestinal polyposis in APCDelta468 mice. *Cancer Res.* 2011;**71**(5):1627–36.
12. Iranshahi M, Sahebkar A, Takasaki M, Konoshima T, Tokuda H. Cancer chemopreventive activity of the prenylated coumarin, umbelliprenin, in vivo. *Eur J Cancer Prev.* 2009;**18**(5):412–5.
13. Barthomeuf C, Lim S, Iranshahi M, Chollet P. Umbelliprenin from Ferula szowitsiana inhibits the growth of human M4Beu metastatic pigmented malignant melanoma cells through cell-cycle arrest in G1 and induction of caspase-dependent apoptosis. *Phytomedicine.* 2008;**15**(1-2):103–11.
14. Khaghanzadeh N, Mojtahedi Z, Ramezani M, Erfani N, Ghaderi A. Umbelliprenin is cytotoxic against QU-DB large cell lung cancer cell line but anti-proliferative against A549 adenocarcinoma cells. *Daru.* 2012;**20**(1):69.
15. Jabrane A, Ben Jannet H, Mighri Z, Mirjolet JF, Duchamp O, Harzallah-Skhiri F, et al. Two new sesquiterpene derivatives from the Tunisian endemic Ferula tunetana Pom. *Chem Biodivers.* 2010;**7**(2):392–9.
16. Ziai SA, Iranshahi M, Zarnani AH, Jeddi-Tehrani M. Umbelliprenin Induces Apoptosis in CLL Cell Lines. *Iran J Pharm Res.* 2012;**11**(2):653–9.
17. Fakih M. Targeting mechanisms of resistance to anti-EGF receptor therapy in KRAS wild-type colorectal cancer: the path to more personalized medicine. *Future Oncol.* 2013;**9**(4):551–60.