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Antiproliferative Effects of Different Concentrations of Auraptene on MCF7 Cancer Cell

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Abstract

Background: Auraptene is a coumarin derivative extracted from citrus species, such as lemon, grapefruit, and orange. To date, auraptene has shown antioxidant, antibacterial, anti-inflammatory, antiproliferative, antiapoptotic, and antitumor activities. Among these, antitumor activity has become more important over the recent years while its underlying mechanism is not fully understood. The current study was conducted to evaluate the antiproliferative effect of auraptene and its mechanisms on MCF7 cell line.

Method: This experimental study investigated whether hesperidin affected the proliferation of MCF-7 human
breast cancer cells. MCF7 cells were cultured in DMEM medium with 10% fetal bovine serum, 100 µg/ml streptomycin, and 100 units/ml penicillin. The cells were incubated in order to be treated with different concentrations of auraptene and time points. Subsequently, the amount of cytotoxicity and apoptosis was measured utilizing MTT and PI staining.

Results: The MTT assay revealed that auraptene had a significant effect on cell viability and induced apoptosis in MCF7 cells at concentrations of 75, 100, 130, 170, and 200 µM.

Conclusion: In this study, through the induction of apoptosis, auraptene prevented the growth and inhibited the proliferation of MCF7 cells at high concentrations in a dose-dependent manner. However, further investigation is needed to reveal the mechanisms of auraptene concerning apoptosis induction.

Keywords: Auraptene, Apoptosis, Cytotoxicity, Cancer, MCF7 cells, MTT

Introduction

Breast cancer is one of the most prevalent cancers among women.[1] According to the statistics by WHO (World Health Organization), about 2.3 million women develop breast cancer every year.[2] Current therapies are radiotherapy and chemotherapy which can play pivotal roles in the preventation of cell growth or cell death initiation through apoptotic or non-apoptotic mechanisms, necrosis for instance.[3-5] Despite their beneficial effects in cancer treatment, these therapies are accompanied by various side effects,[6] such as leukemia, blood clotting, nausea, and vomiting.[1] Like chemotherapeutic agents, traditional herbal extracts have shown similar toxic effects on cancer cells, yet fewer side effects; hence, their usage instead of chemical drugs is recommended.[7]

A great deal of evidence has indicated that the consumption of fruits and vegetables in the diet is associated with a reduction in the risk of cancer incidence owing to their chemical compounds able to inhibit or reverse the development of cancer.[8] Among these natural compounds, we could mention auraptene which is extracted from citrus fruits, such as lemon, grapefruit, and orange.[9] Auraptene (7-geranyloxycoumarin) is a well-known and the most abundant prenyloxycoumarin compound in various plant species, including the Rutaceae and Umbelliferae (Apiaceae) families.[8]

Auraptene has a significant effect on the prevention of degenerative diseases. Owing to its antimicrobial and anti-inflammatory properties, it has been used to control the periodontal diseases. It is also of anti-inflammatory, antiproliferative, and antiapoptotic properties.[10,11] Auraptene has demonstrated a protective effect against cardiovascular diseases and oxidative stress-associated disorders.[12,13] In account of its antioxidant feature, auraptene protects DNA and other cellular macromolecules against free radicals and has shown a protective effect on different organs, such as liver, skin, esophagus, and colon, and also prevents inflammation and metabolic disorders.[12,13] Auraptene has shown its effectiveness as a chemotherapeutic agent through various mechanisms, including inhibition of polyamine synthesis, inhibition of metalloproteinase activity, induction of anti-detoxification enzymes and apoptosis.[12,13]

Materials and Methods

This experimental study (Lab trial) was done based on a lab trial; it could be considered as a sub group of experimental studies.

Chemicals and reagents

Auraptene, which was synthesized through a particular mechanism, was kindly prepared by the professors of Sabzevar University of Medical Sciences. Dulbecco's phosphate-buffered saline (PBS) and 4, 5-dimethylimidazole-2-yl, 2, 5-diphenyl tetrazolium (MTT) were purchased from Sigma (St Louis, MO, USA). Glucose-high Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were purchased from Gibco (Grand Island, NY). Dimethyl sulfoxide (DMSO) was purchased from Merck (Germany). Propidium iodide (PI), sodium citrate, and Triton X-100 were purchased from Sigma (St Louis, MO, USA).

Cell culture

The cells were primarily cultured in DMEM with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/ml streptomycin supplemented with 5% CO_2 at 37°C. They were plated and incubated with various concentrations of auraptene for 24, 48, and 72 h. For MTT assay, the cells were seeded at 5×10^3/well into a 96-well culture plate. For each concentration and time course, a control sample was employed. For apoptosis

assessment, the cells were seeded at 1×10^5/well into a 24-well plate. All the experiments were carried out in triplicate.

Cell viability

The cell viability was determined with MTT assay.[14] Briefly, MCF7 cells were seeded at 5×10^3/well in flat-bottom 96-well culture plates and incubated for 24h. After that, cells were incubated with various concentrations of auraptene from 0.1 up to 200 µM for 24, 48 and 72 h. After removing the medium, the cells were incubated with MTT solution (5mg/ml in PBS) for 4h and the obtained formazan was solubilized with DMSO (100 µl). The absorption was measured at 570 nm (620 nm as a reference) using an ELISA reader. Later, the IC50s were determined utilizing Prism software. All the treatments were carried out in triplicate.

Cell apoptosis assay

Based on PI staining of small DNA fragments, apoptotic cells were detected using flow cytometry. It has been reported that a sub-G1 peak reflects DNA fragmentation following the incubation of cells with a hypotonic phosphate-citrate buffer containing a quantitative DNA-binding dye, such as PI (15). Briefly, MCF7 cells were seeded in a 24-well plate and treated with concentrations of 36 and 21.66 µM (IC50) of auraptene for 48h and 72h, respectively. Afterwards, the floating and adherent cells were harvested and incubated with 750 µL of a hypotonic buffer, 50 mg/ml PI in 0.1% sodium citrate with 0.1% Triton X-100, at 4 °C overnight in the dark. Finally, flow cytometry was carried out using a FACScan flow cytometer (Becton Dickinson). A total of 1×10^4 events were acquired with FACS and the data was analyzed with flowJo-V10 software. All the treatments were carried out in triplicate.

Statistical analysis

All the obtained results are expressed as mean ± SEM. The significance of difference was evaluated with ANOVA and Bonfrroni's test. A probability level of $P<0.05$ was considered to be statistically significant.

Results

The effect of auraptene on the viability of MCF7 cells

The results of MTT depicted that the viability of the MCF7 cells is dose-dependent; it decreased in higher concentrations (<75 µM) of auraptene (Figure 1). At the exposure time of 24h with the concentrations of 75, 100, 130, 170, and 200, auraptene had a significant effect on decreasing cells viability ($P< 0.001$). At the exposure times of 48 and 72h with the concentrations of 30, 50, 75, 100, 130, 170, and 200, it had a significant effect on reducing cells viability ($P< 0.001$). These results indicated that the treatment of MCF7 cells with auraptene was dose-dependent.

The assessment of apoptotic MCF7 cells using PI staining

The toxic effects of auraptene on the MCF7 cells were also observed. The concentrations of IC50 were calculated for 48 and 72h, which were 36 and 21.66, respectively. The amount of apoptosis cell was measured employing PI staining. According to Figure 2 and Figure 3, in the auraptene treated groups, the sub-G1 peak analysis indicated the amount of apoptosis induction in the MCF7 cells.

Discussion

Herein, we found that auraptene had an inhibitory effect on the MCF7 cells by decreasing cells viability in a dose-dependent manner. It is noteworthy that in higher concentrations, considerable decreases were reported in cell viability of MCF7.

The PI staining results implied that auraptene raised a peak at sub-G1, which showed DNA fragmentation as an apoptotic

property compared to the control group in the MCF7 cells. Previous studies have shown similar effects; Umbelliprenin as a similar component of Ferulla family has antiproliferative effects on Jurkat cells.[17] Auraptene decreases cell viability by inducing apoptosis in the colon carcinoma. For note, our data were confirmed with MTS assay as an in vitro assay for cell proliferation and DNA fragmentation assay.[18] Based on previous results, analysis of gene expression in natural fibroblasts has shown that auraptene in higher concentrations not only decreases bcl-2 expression, but also increases Bax and Caspase-3 expression.[16] Similarly, previous reports have indicated that auraptene induced apoptosis in prostate cancer cells via Mcl-1-mediated activation of caspases.[16] In addition, studies have reported that auraptene induces apoptosis in Jurkat cells by caspase-8 activation. In other words, the pathways through which apoptosis occurs due to the activation of caspase cascade are independent or dependent on the mitochondrial pathway.[19] One of the important mechanisms of auraptene is known to be cell cycle arrest in the G1/S phase.[20] Auraptene, by raising a peak at sub-G1, which shows DNA fragmentation, induces inhibitory effects owing to the onset of apoptosis.[21] Therefore, auraptene reduces the MCF7 cells in the S phase after IGF-1treatment for 24 h, and also the mRNA levels of genes, which promote G1/S transition and DNA replication.[11] It significantly reduces the expression of iNOS/COX-2 and the release of TNF-α.[22] Auraptene reduces Cyclin D1 protein expression following the treatment with IGF-1.[23] Since Cyclin D1 is a key protein that regulates G1/S transmission in cell cycle,[24] auraptene is assumed to inhibit the progression of the cell cycle by inhibiting the transcription of S phase genes,[23] n view of the presence of its active metabolites, for instance, umbelliferone and 7-ethoxycoumarin with chemopreventive effects.[25]

In sum, several studies have reported various effects of auraptene and its derivatives. Our results revealed that auraptene inhibited the proliferation of the MCF7 cells at high concentrations, and also induced apoptosis in cancer cells. However, further research is required in order to evaluate the underlying mechanisms by which auraptene induces its apoptotic effects.

Conflict of Interest

None declared.

References

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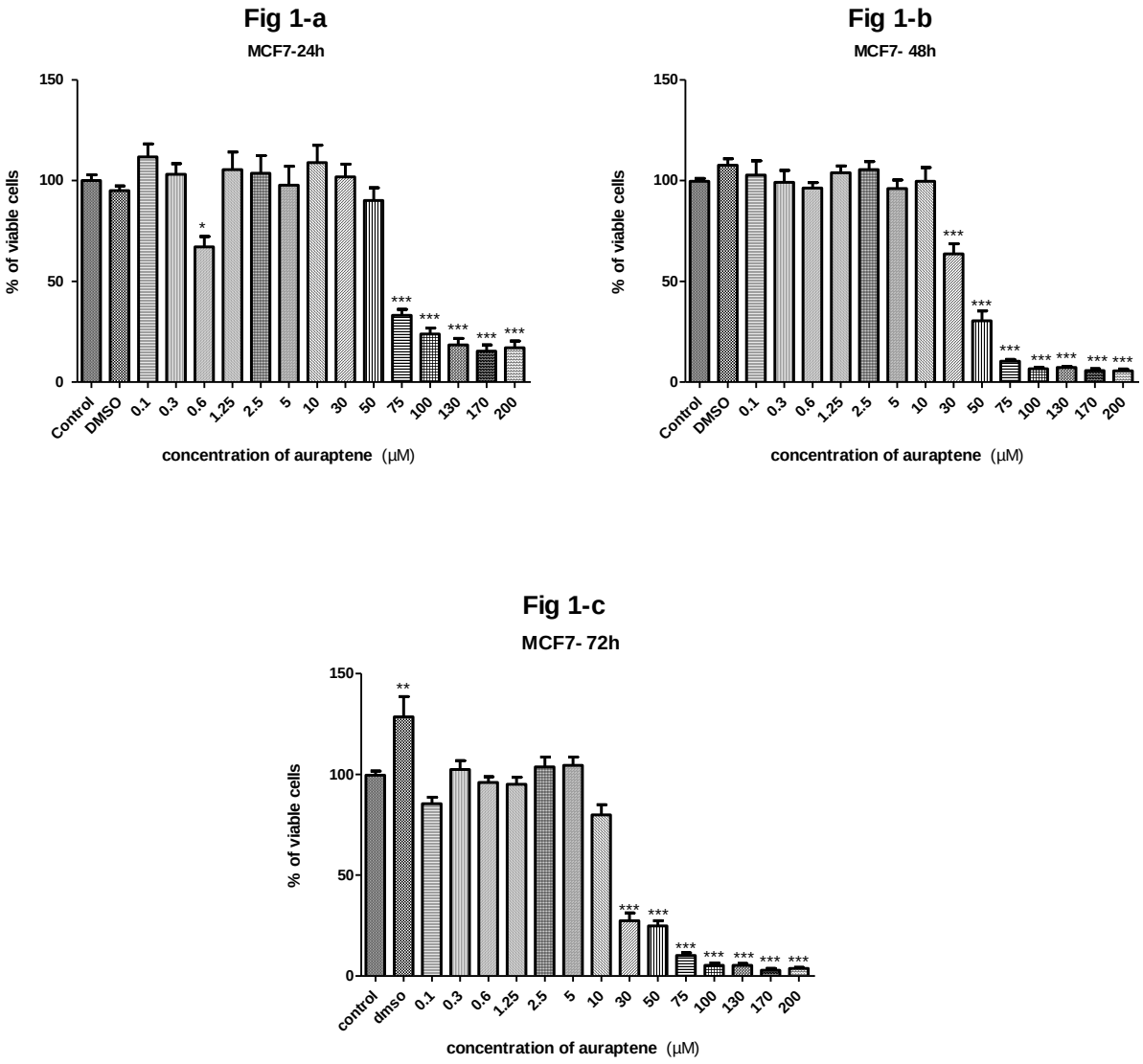

Figure 1. The effect of auraptene on the cell viability of MCF7 cells. Cell viability of MCF7 cells was measured with the MTT assay. The cells were treated with different concentrations of auraptene (0.1-200) for 24 (a), 48 (b), and 72h (c). The highest inhibitory effects were observed at the concentrations of 75, 100, 130, 170, and 200. The exposures of 48 and 72h were more effective on viability reduction. IC50s for 48 and 72h treatment were calculated; they were 36 and 21.66 μM, respectively. The results are expressed as Mean ± SEM (n = 3). * P<0.05 , *** P< 0.0001.

(IC_{50}): half maximal inhibitory concentration

Fig2-a

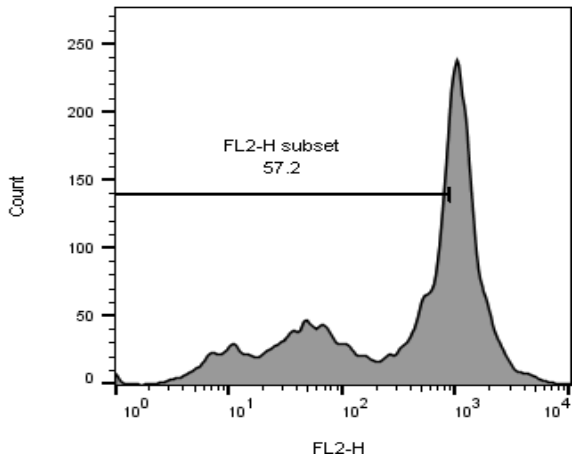

Fig2-b

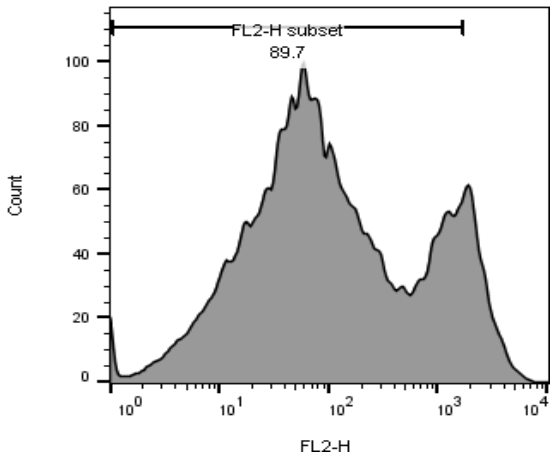

Fig 2-c

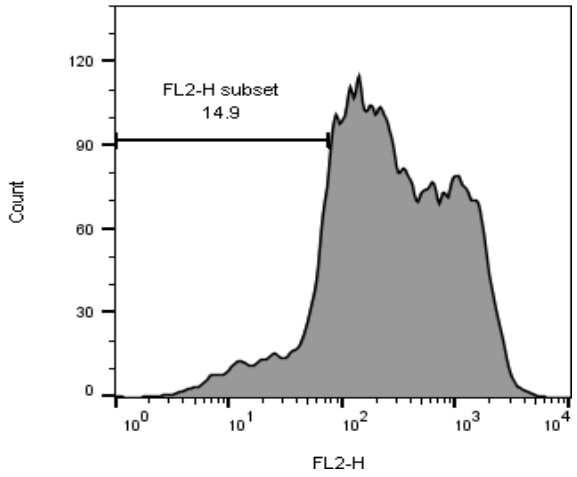

Fig 2-d

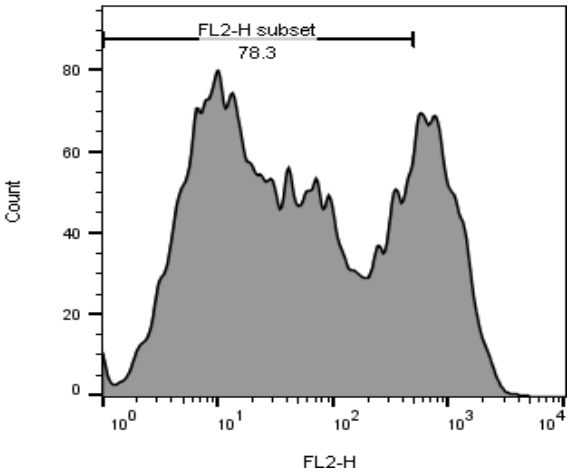

Figure 2. Auraptene induced apoptosis on the MCF7 cells. (a) flow cytometry histogram of PI-staining on MCF7 cells in the control group for 48h. (b)flow cytometry histogram of PI-staining on the MCF7 cells treated with a concentration of 36 µM of auraptene for 48 h.(c) flow cytometry histogram of PI-staining on the MCF7 cells in the control group for 72h. (d) flow cytometry histogram of PI-staining on the MCF7 cells treated with a concentration of 21.66 µM of auraptene for 72 h.

PI: Propidium iodide

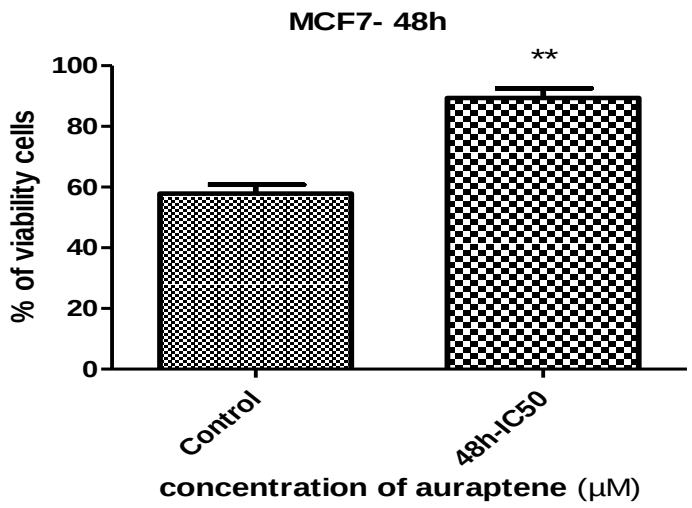

Fig 3-a

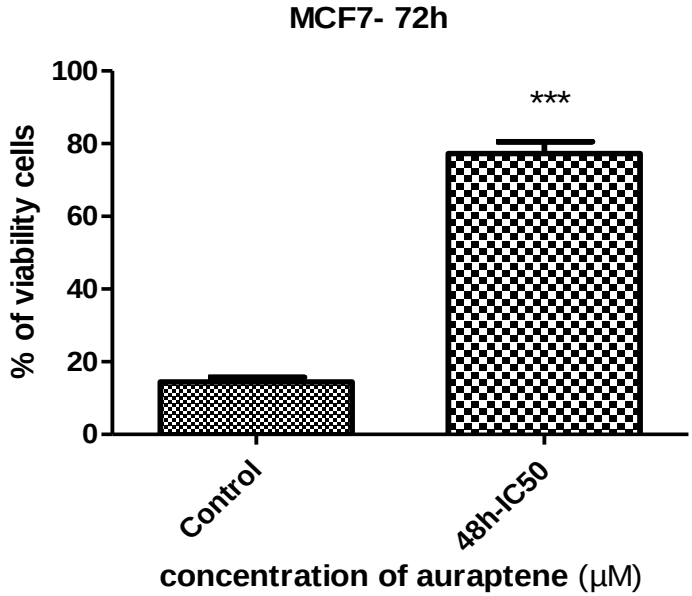

Fig 3-b

Figure 3. PI-staining of apoptotic the MCF7 cells. (a) The MCF7 cells were treated with concentration of 36 μM of auraptene for 48h. (b) The MCF7 cells were treated with concentration of 21.66 μM of auraptene for 72h. It should be noted that these concentrations were IC50s for 48h and 72h. $^{**}P < 0.01$,$^{***}P < 0.0001$.
PI: Propidium iodide