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Apoptosis-Inducing Effect of Hesperidin on Breast Cancer Cell Line MCF7

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Abstract

Hesperidin is a flavanone present in citrus fruits, such as oranges and lemons. It exerts non-toxic activities in normal cells; however, it has been reported to suppress cell proliferation in several cancer types. Moreover, it was shown that dietary hesperidin acts as an anti-carcinogenic agent for some tumors. In the present study, we investigated the effect of hesperidin on breast cancer cell line MCF7 and also its effects on the expression of apoptosis-related genes in this cell line.

MCF7 cells were divided into 4 groups, including 3 study groups and 1 control group. Each study group was treated with 50, 75, or 100 µg/mL hesperidin, while the control group was left untreated. The samples underwent real-time PCR using the primers specific for *BCL-2* and *BAX*, as the study genes, while GAPDH was used as the control.

According to our findings, hesperidin caused a significant decrease in BCL-2 mRNA levels at all the doses used in the study groups compared to the control group ($p < 0.002$). The observed decrease was dose-dependent. Also, hesperidin induced a significant overexpression of *BAX* when used in doses of 75 and 100 µg/mL in comparison to the control group.

The present study proved the significant apoptosis-inducing effect of hesperidin on the breast cancer cell line MCF7.

INTRODUCTION

Hesperidin is a flavanone present in citrus fruits, such as oranges and lemons. It was first discovered in 1827 but had been studied as a combination product complex until 1986 (1). In nature, it is found in the glycoside form, which is a beta-7-rutinoside of hespetidin; however, dietary hesperidin is hydrolyzed to hesperitin. Hesperidin has non-toxic activities in normal cells, although it has been reported to suppress cell proliferation in several cancer types (2). Moreover, dietary hesperidin acts as an anti-carcinogenic agent for some tumors. Similar to flavonoids, hesperidin is known to have anti-inflammatory, anti-viral, UV-protecting, anti-oxidant, pro-apoptotic, anti-proliferative, and anti-tumor properties. Also, it has protective effects against cerebrovascular diseases and diabetes mellitus (3). As a radioprotective and chemoprotective therapeutic agent, it is expected to prevent invasion or metastasis of human cancers (4). Anti-cancer effects of hesperidin have been studied in tumor-implanted animal models or culture cell lines of several cancer types, including colon cancer, bladder cancer, hepatocarcinoma, and breast cancer. However, the action mechanism of this compound is not fully understood (5). Also, it was reported that hesperidin triggered apoptosis through the extrinsic pathway and induced cell cycle arrest via the endoplasmic reticulum stress pathway in HeLa cells. Saiprasad et al. observed that hesperidin initiated apoptosis and autophagy through mediating Aurora-A-coupled pro-survival phosphoinositide 3-kinase/Akt/mammalian target of rapamycin signaling cascades and glycogen synthase kinase-3β activity to antagonize the effect of azoxymethane on colon carcinogenesis in a mouse model (6). In the present study, we investigated the effect of hesperidin on breast cancer cell line MCF7 and also its effects on the expression of apoptosis-related genes in this cell line.

METHODS AND MATERIALS

The National Cell Bank (Pasteur Institute, Iran) supplied the human MCF-7 breast cancer cells. The

cells were then cultured in RPMI 1640 media (Sigma, St Louis, MO, USA) containing 10% fetal bovine serum (FBS; Invitrogen), streptomycin (100 mg/mL), and penicillin (100 units/mL) (Sigma, St Louis, MO, USA). Next, the cells were incubated (37 °C) in a humidified ambiance with 5% CO_2. Hesperidin was dissolved in DMSO to produce a 25 mg/mL stock solution and stored as aliquots in tightly sealed vials at -20° C. Working solutions were prepared by serial dilutions of stock solution with whole culture medium. Thereafter, the cells were divided into 4 groups:

- Group 1: non-treated group as the control group
- Group 2: 50 µg/mL hesperidin
- Group 3: 75 µg/mL hesperidin
- Group 4: 100 µg/mL hesperidin

Total RNA was isolated from the cells using the RiboEX total RNA solution (GeneAll, Korea). 2 mg of RNA was used to synthesize cDNA using the ExcelRT™ Reverse Transcription Kit II (SMOBIO, Taiwan). Real-time PCR was performed using the specific primers for *BCL*-2 and BAX, as the study genes, and GAPDH, as the control gene (Table 1). Data were analyzed using the 2^-ΔΔct method.

RESULTS

According to our findings, the control group showed no significant increase. Figure 1 presents the effect of hesperidin at doses 50, 75, and 100 µg/mL on the expression levels of *BCL-2* and *BAX* in the MCF7 cell line. As illustrated, hesperidin caused a significant decrease in BCL-2 mRNA levels at all the doses used in the study groups compared to the control group ($p < 0.002$). The observed decrease was dose-dependent. Also, hesperidin induced a significant overexpression of *BAX* when used in doses of 75 and 100 µg/mL in comparison to the control group.

Table 1. Primer sequences

Primer name	Primer sequence
GAPDH	F:5'- CTGGGCTACACTGAGCACC -3' R:5'- AAGTGGTCGTTGAGGGCAATG -3'
BAX	F:5'- CCCGAGAGGTCTTTTTCCGAG -3' R:5'- CCAGCCCATGATGGTTCTGAT -3'
BclBCL-2	F:5'- CCTATCTGGGCCACAAGTGAA -3' R:5'- ACAGCCTGCAGCTTTGTTTC -3'

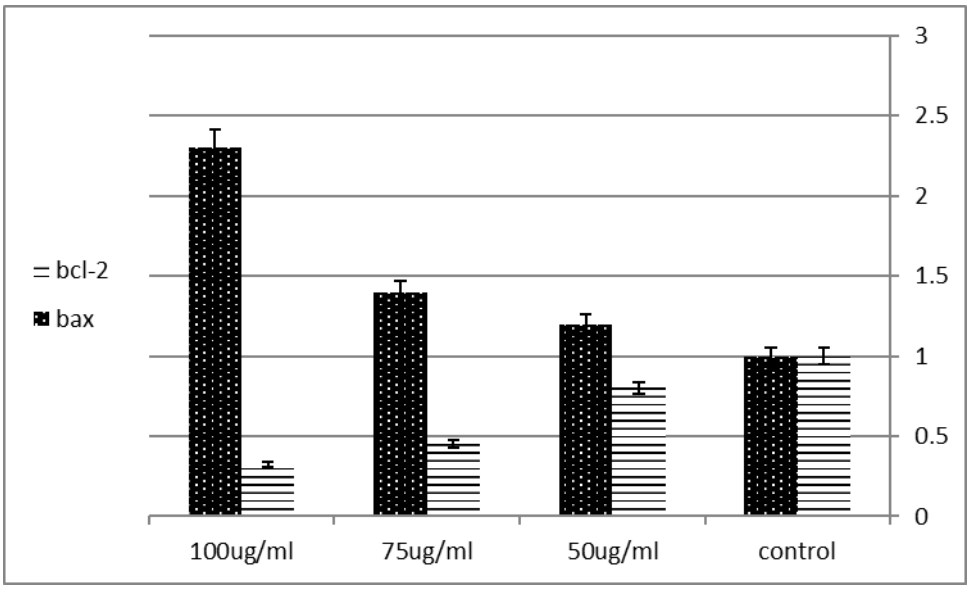

Fig 1. BAX and BCL-2 mRNA expressions by group

DISCUSSION

The present study proved the significant apoptosis-inducing effect of hesperidin on the breast cancer cell line MCF7. Tumor occurrence and development are associated not only with dedifferentiation and excessive multiplication of tumor cells, but are also with the suppression of apoptosis (10). To investigate the mechanisms underlying the effect of hesperidin on apoptosis, apoptosis-associated signaling molecules in MCF7 cells were detected. Then, it was demonstrated that cell apoptosis was increased after treatment with hesperidin (11). Apoptosis is Apoptosis is regulated by multiple apoptosis-promoting proteins, such as BAX, BAD, and BID and is up-regulated by a family

of apoptosis-inhibiting proteins, including BCL-2 and BCL-xL (12). The balance between apoptosis-promoting and apoptosis-inhibiting proteins is crucial for the process of apoptosis (13). The present study results showed that treating the MCF7 cells with hesperidin could up-regulate the relative levels of BAX mRNA while down-regulating the relative expression levels of BCL-2 mRNA. These findings proved that MCF7 cell apoptosis was induced by hesperidin via activation of the apoptotic pathway (14). Flavonoids are studied as anti-cancer agents for chemotherapy in cancer treatment because of their advantages, such as being non-toxic and natural. Representative flavonoids, such as resveratrol, quercetin, curcumin, and EGCG, have been studied for this purpose in clinical trials (15-17). However, they have not yet been developed for clinical treatments in cancer therapy. Hesperidin and related metabolites have significant potential as therapeutic agents for a wide range of diseases and disorders.

REFERENCE

1. NSCLC Meta-analysis Collaborative Group: Preoperative chemotherapy For non-small-cell lung cancer: A systematic Review and meta-analysis of individual participant data. Lancet383:1561-1571, 2014.

2. Saintigny P and Burger JA: Recent advances in non-small cell lung cancer biology and clinical management. Discov Med 13: 287-297, 2012.

3. He J, Shen J, Yang C, Jiang L, Liang W, Shi X, Xu X and He J: Adjuvant chemotherapy for the completely resected stage IB nonsmall cell lung cancer: A systematic review and meta-analysis. Medicine (Baltimore) 94: e903, 2015.

4. DeSantis CE, Lin CC, Mariotto AB, Siegel RL, Stein KD, Kramer JL, Alteri R, Robbins AS and Jemal A: Cancer treatment and survivorship statistics, 2014. CA Cancer J Clin 64: 252-271, 2014.

5. Singh BN, Singh HB, Singh A, Naqvi AH and Singh BR: Dietary phytochemicals alter epigenetic events and signaling pathways for inhibition of metastasis cascade: Phytoblockers of metastasis cascade. Cancer Metastasis Rev 33: 41-85, 2014.

6. Lu Y, Zhang C, Bucheli P and Wei D: Citrus flavonoids in fruit and traditional Chinese medicinal food ingredients in China. Plant Foods Hum Nutr 61: 57-65, 2006.

7. Roohbakhsh A, Parhiz H, Soltani F, Rezaee R and Iranshahi M: Molecular mechanisms behind the biological effects of hesperidin and hesperetin for the prevention of cancer and cardiovascular diseases. Life Sci 124: 64-74, 2015.

8. Knekt P, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, Hakulinen T and Aromaa A: Flavonoid intake and risk of chronic diseases. Am J Clin Nutr 76: 560-568, 2002.

9. Ahmadi A, Shadboorestan A, Nabavi SF, Setzer WN and Nabavi SM: The role of hesperidin in cell signal transduction pathway for the prevention or treatment of cancer. Curr Med Chem 22: 3462-3471, 2015.

10. Tanaka T, Tanaka T, Tanaka M and Kuno T: Cancer chemoprevention By citrus pulp and juices containing high amounts of β-cryptoxanthin and hesperidin. J Biomed Biotechnol 2012:516981, 2012.

11. Yumnam S, Hong GE, Raha S, Saralamma VV, Lee HJ, Lee WS, Kim EH and Kim GS: Mitochondrial dysfunction and Ca(2+) overload contributes to hesperidin induced paraptosis in hepatoblastoma cells, HepG2.J Cell Physiol231: 1261-1268,2016.

12. Banjerdpongchai R, Wudtiwai B, Khaw-On P, Rachakhom W, Duangnil N And Kongtawelert P:Hesperidin From Citrus Seed induces human hepatocellular carcinoma HepG2 Cell apoptosis via both mitochondrial and death receptor pathways. Tumour Biol 37: 227-237, 2016.

13. Yumnam S, Park HS, Kim MK, Nagappan A, Hong GE, Lee HJ, Lee WS, Kim EH, Cho JH, Shin SC, Et al: Hesperidin Induces paraptosis like cell death in hepatoblastoma, HepG2 cells: Involvement Of ERK1/2 MAPK [corrected].PLoS One 9: e101321, 2014.

14. Ghorbani A, Nazari M, Jeddi-Tehrani M and Zand H: The citrusflavonoid hesperidin induces p53 and inhibits NF-κB Activation in order to trigger apoptosis in NALM-6 cells: Involvement Of PPARγ-dependent mechanism. Eur J Nutr 51: 39-46, 2012.

15. Nazari M Ghorbani A, Hekmat-Doost A,Jeddi-Tehrani M andZand H: Inactivation Of nuclear factor-κB by citrus flavanone hesperidin contributes to apoptosis and chemo-sensitizing effect in Ramos cells. Eur J Pharmacol650:526-533, 2011.

16. Lee KA, Lee SH, Lee YJ, Baeg SM And Shim JH: Hesperidin Induces apoptosis by inhibiting Sp1 And its regulatory protein in MSTO-211H cells. Biomol Ther (Seoul)20: 273-279, 2012.

17. Bartoszewski R, Hering A, Marszałł M, Stefanowicz Hajduk J, Bartoszewska S, Kapoor N, Kochan K and Ochocka R: Mangiferin Has an additive effect on the apoptotic properties of hesperidin in Cyclopia sp.Tea extracts PLoS One 9: e92128,2014.