Northwestern Med J.2022;2(1):16-22
doi: 10.54307/NWMJ.2022.28247

Original Article

Cytotoxic effects of phloridzin

Phloridzin'in sitotoksik etkisi

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Atıf/Cite as: Kilit AC, Aydemir E. Cytotoxic effects of phloridzin. Northwestern Med J.2022;2(1):16-22.

ABSTRACT

Cancer has been the most lethal disease globally, notably in the last century. Each type of cancer and the response of cancer cells to treatment is unique motivates scientists to conduct new studies every day. Due to accumulating mutations, existing drugs, particularly those intended to treat cancer, are insufficient in treating cancer. This creates a strong need for research to discover new molecules or increase drug efficacy in cancer treatment. Regarding the development of alternative anticancer drugs, plant-derived agents stand out for their correlation with cancer. The flavonoids included in the fruits and vegetables we eat daily are thought to play a role in cancer prevention and provide new therapeutic options. Flavonoids are thermostable polyphenolic compounds commonly found in nature. In our study, the Phloridzin flavonoid obtained from apples, a fruit that we consume a lot in our daily life, has been tested on different cell lines such as MDAMB231, MCF-7, 293T, 22RV1, U87, A549, and it has been tested whether it has a cytotoxic effect. We observed that Phloridzin exhibited selective cytotoxicity in these cancer cells. We hope that this research will shed light on further research on the mechanism of death of Phloridzin and its use as an alternative to cancer treatment.

Keywords: cancer, phenolic compounds, phloridzin, cytotoxicity, MDAMB231, MCF-7, 22RV1, U87, A549

ÖZ

Kanser özellikle son yüzyılda tüm dünyanın en çok ölümle sonuçlanan hastalığıdır. Her kanser tipinin ve kanser hücrelerinin tedaviye verdiği yanıtın farklı oluşu bilim insanlarını da her gün yeni araştırmalar yapmaya yöneltmektedir. Özellikle kanser tedavisi için kullanılan mevcut ilaçlar, birikerek çoğalan mutasyonlar nedeniyle kansere karşı tedavide yetersiz kalmaktadır. Bu nedenle kanser tedavisinde yeni molekülleri keşfetmeye veya ilaç etkinliğini artırmaya yönelik yapılan çalışmalara ihtiyaç vardır. Alternatif antikanser ilaçların geliştirilmesi açısından bakıldığında bitkisel türevli ajanların kanserle olan ilişkileri göze çarpmaktadır. Beslenmeyle günlük diyetimizde aldığımız meyve ve sebzelerde bulunan flavonoidlerin, kanserin önlenmesinde önemli bir rol oynayabileceği ve tedavilere ek kaynaklar olabilecekleri düşünülmektedir. Flavonoidler, doğada yaygın olarak bulunan, ısıya dayanıklı polifenolik bileşiklerdir. Çalışmamızda günlük yaşantımızda çokça tükettiğimiz elmadan elde edilen Phloridzin flavonoidi MDAMB231, MCF-7, 293T, 22RV1, U87, A549 gibi farklı hücre hatları üzerinde denenmiş olup, sitotoksik etki gösterip göstermediği test edilmiştir. Phloridzinin bahsi geçen kanser hücrelerinde seçici sitotoksisite gösterdiği gözlemlenmiştir. Bu çalışmanın Pholoridzinin daha ayrıntılı ölüm mekanizmasının araştırılması ve kanser tedavilerine alternatif tedavi olabilmesi konusunda diğer çalışmalara ışık tutacağını umut etmekteyiz.

Anahtar kelimeler: kanser, fenolik bileşikler, phloridzin, sitotoksisite, MDAMB231, MCF-7, 22RV1, U87, A549

Received: 08.11.2021
Accepted: 01.03.2022
Publication date: 01.18.2022

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INTRODUCTION

Cancer is described as the progression of damaged cells through the cell cycle, which should typically die, due to the accumulation of functional abnormalities and mutations in the cell cycle's control (1). Cancer is a systemic disease that occurs with many mutations in the genome. Although it is defined as a genetic disease caused by the deterioration of the information in DNA, which is the genetic material of the cell, when cancer cases are examined, it is observed that there are abnormal expressions in gene expressions. Additionally, epigenetic modifications that are not caused by mutations appear to be significant factors (2,3). When a healthy cell encounters a pathological condition, the cell is driven to death by apoptosis, autophagy, and programmed necrosis, all of which are known as programmed cell deaths. These three types of programmed death work together to determine the cell's fate (4).

Due to drug resistance and cumulative mutations, conventional cancer treatments used in the standard treatment are insufficient in cancer therapy. This constitutes a strong need for studies to discover new molecules or increase drug efficacy in cancer treatment. Recent studies have contributed to introducing many potential chemotherapeutic agents to the market for developing new approaches against cancer. These agents have been successfully used in clinical applications. However, due to the side effects of existing chemotherapeutic agents and multidrug resistance, there is a need for new agents that can be used as alternatives or supplements to these. The common goal of all researches in this field is to discover and adopt therapeutic agents that have selective cytotoxic effects in cancer cells while causing no harmful responses in normal cells. Therefore, developing new alternative agents that can be considered effective and safe is critical for cancer treatment. At this point, we encounter plant-derived extracts or metabolites isolated from plants (5).

Plant-derived natural products stand out as promising and potent new chemotherapeutic agent candidates for cancer treatment thanks to their lower costs and fewer side effects. Flavonoids are especially attractive among these phytochemicals because of their diverse biochemical and pharmacological characteristics (6).

Regarding new anticancer drugs in terms of design, the correlation between plant-derived agents and cancer stands out, and researchers are drawn to this field because each of these agents has potential. Herbs have been demonstrated to be effective in treating cancer in numerous scientific studies. Examining natural compounds acquired from plants pharmacologically, elucidating the mechanisms that provide such effects, and determining the components responsible for these effects can guide research on this matter. Flavonoids are among the natural compounds tried in many clinical trials (5,7,8). They are also natural polyphenols found in various plants and are frequently seen as secondary metabolites with a low molecular weight (9). Reports indicate that they have many biochemical and pharmacological effects, including antioxidant, anti-inflammatory, anticancer, antimicrobial, and immunomodulatory activities (10-12).

Phloridzin, a phenolic compound (also called phloretin 2'-O-glucoside, phlorizin, phlorrhizin, phlorhizin, or phlorizoside), is a prominent member of the class of dihydrochalcones, which are phenylpropanoids (13). The apple tree (Malus spp.) contains abundant Phloridzin flavonoids in its structure. Phloridzin and its derivatives have been widely used in biological, medical and physiological research for almost 200 years since their discovery. The studies show that it is especially related to diabetes, obesity, stress hyperglycemia, antioxidant activity, membrane permeability and long-lasting agents used in the food industry (especially in food additives), pharmaceuticals and cosmetics (14-18). In addition to these studies, several suggest that Phloridzin may act as an anticancer agent (19-23).

MATERIALS AND METHODS

Cells and culture conditions

MDA-MB-231 (ATCC® HTB-26™ estrogen receptor-negative human breast cancer cell line), MCF-7 (ATCC® HTB-22™, estrogen receptor-positive human breast cancer cell line), PANC-1 (ATCC® CRL-1469™ human pancreatic cancer epithelial cell line), U87 (ATCC® HTB-14™, human brain cancer epithelial cell line), A549 (ATCC® CCL-185™ human non-small cell lung cancer cell line), 22RV-1 (ATCC® CRL-2505™ human prostate cancer epithelial cell line), and 293T (ATCC® CRL-1573™ human kidney epithelial cell line) were obtained from the Cancer Molecular Biology laboratory at Akdeniz University, Biology Department, opened in cell culture under appropriate conditions and grown in an RPMI 1640 medium. Enough passages were obtained to reach sufficient numbers for the experiment. In the vacuum furnace, all cell lines were incubated in a 5% CO_2 atmosphere at 37°C. The cells were lifted using a mixture of 0.25% trypsin, 0.03% EDTA, as recommended by ATCC, and passaged at a ratio of 1:2 or 1:3. Unused cells were stored in environments of 95% medium and 5% DMSO-containing freezing medium at -80°C.

Applying phloridzin flavonoid to cells

Phloridzin flavonoid was obtained purely from TransMIT (PlantMetaChem- P-012), dissolved in serum-free medium, and stocked in RPMI medium as 2 mg/mL stocks. Cells were seeded into small Petri dishes, removed by trypsinisation when the dishes were 80-90% full, and seeded into sterile 96-well plates at $1x10^4$ cells/well. The media were removed after 24 hours. The drugs were added to media containing 1% serum by reducing the doses in half (200-25 µg/mL) by serial dilution, with a maximum dose of 200 µg/mL. The incubation period was determined as 24 hours. This way, we tried to determine the time and dose-dependent cytotoxic effects of Phloridzin on the cell lines.

WST-1 cell proliferation test

(CAYMAN Kat No:1000883) The cytotoxic effect of Phloridzin was investigated using a WST-1 cell proliferation kit. The WST-1 test is based on the principle that the metabolic activity in living cells breaks down WST-1 via the mitochondrial dehydrogenase enzyme, forming soluble formazan salts. In the test, the cells were seeded into small Petri dishes, removed by trypsinisation when the dishes were 80-90% full, and seeded into sterile 96-well plates at $1x10^4$ cells/well. The media were removed after 24 hours, and all doses were prepared in a medium containing 1% FBS (Fetal Bovine Serum) with a maximum dose of 200 µg/ml (200-25 µg/ml) added to the wells. After the 24-hour incubation period, the media were withdrawn, 90 µL of WST-1 solution was added to 10 µL of serum-free media, and allowed to incubate for an average of 4 hours. After incubation, the absorbance values of the plates were measured using a spectrophotometer at a wavelength of 450nm.

Statistical analysis

Differences in cytotoxicity findings between control and other groups were evaluated using the One Way ANOVA Test and the Dunnett's Multiple Comparison Test on the Graph-Pad InStat statistical software. All data are plotted as mean ± SEM using the Sigma Plot 10.0 software.

RESULTS

Cytotoxic effect of phloridzin on MDAMB 231, MCF-7, PANC-1, U87, A549, 22RV1, and 293T cell lines by WST-1 test

The cytotoxic effect of Phloridzin flavonoid on cell lines MDAMB 231, MCF-7, U87, PANC-1, A549, 22RV1, and 293T was tested at doses of 200, 100, 50, and 25 µg/mL in 24-hour incubation periods. Each experiment was repeated four times. In line with the findings, the effect of applying Phloridzin to cell lines MDAMB231 (Figure 1), MCF-7 (Figure 2), PANC-1 (Figure 3), U87 (Figure 4), A549 (Figure 5), 22RV1 (Figure 6), and 293T (Figure 7) in the range of 200-25 µg/mL on cell viability was evaluated and plotted using the Sigmaplot statistics software.

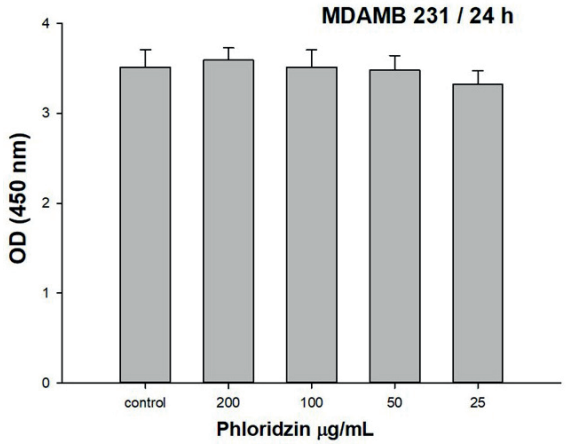

Figure 1. Effect of Phloridzin on cell viability in MDAMB 231 between 200-25 µg/mL over 24-hour incubation.

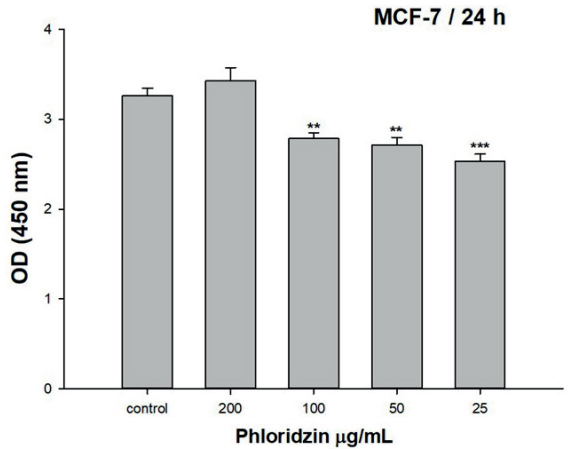

Figure 2. Effect of Phloridzin on cell viability in MCF-7 between 200-25 µg/mL over 24-hour incubation (, p<0.001 ***, p<0.001).**

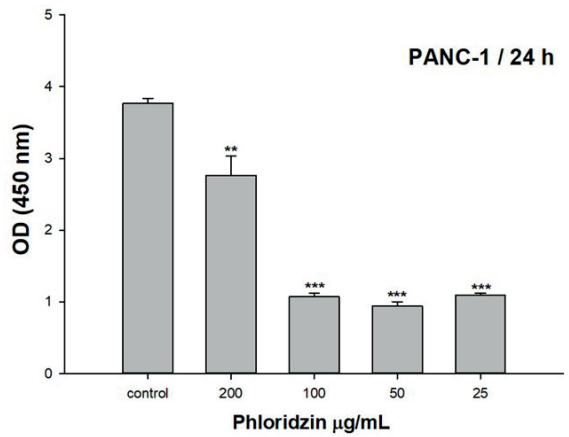

Figure 3. Effect of Phloridzin on cell viability in PANC-1 between 200-25 µg/mL over 24-hour incubation (, p<0.01; ***, p<0.001).**

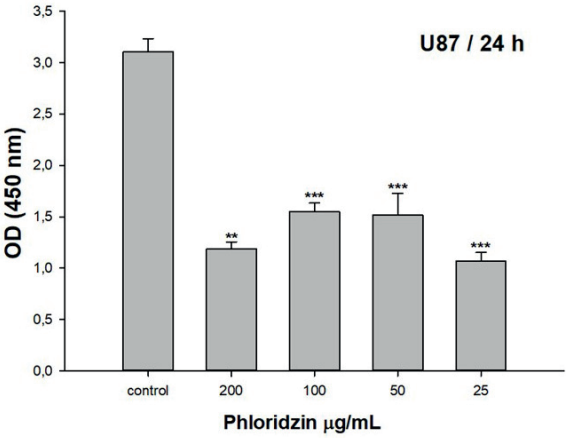

Figure 4. Effect of Phloridzin on cell viability in U87 between 200-25 µg/mL over 24-hour incubation (*, p<0.001; **, p<0.01).**

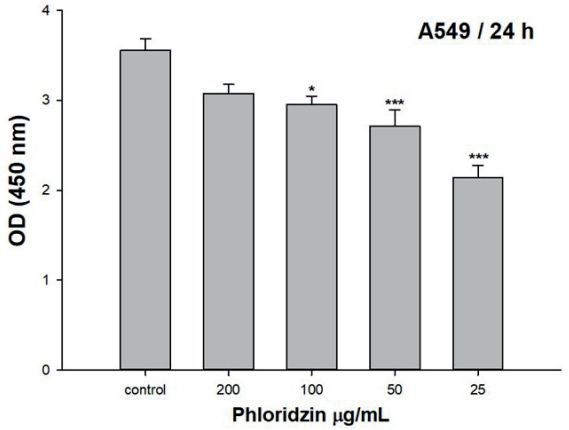

Figure 5. Effect of Phloridzin on cell viability in A549 between 200-25 µg/mL over 24-hour incubation (*, p<0.001; *, p<0.05).**

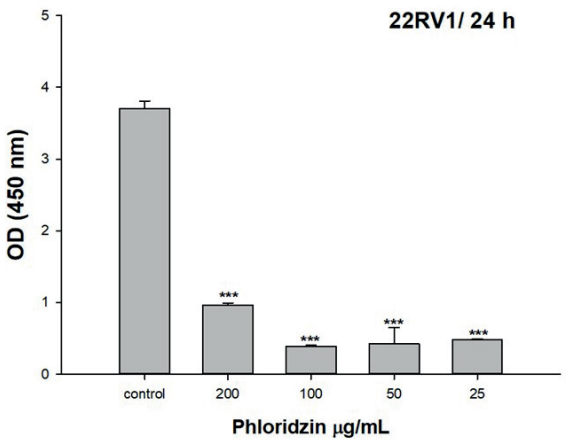

Figure 6. Effect of Phloridzin on cell viability in 22RV1 between 200-25 µg/mL over 24-hour incubation (*, p<0.001).**

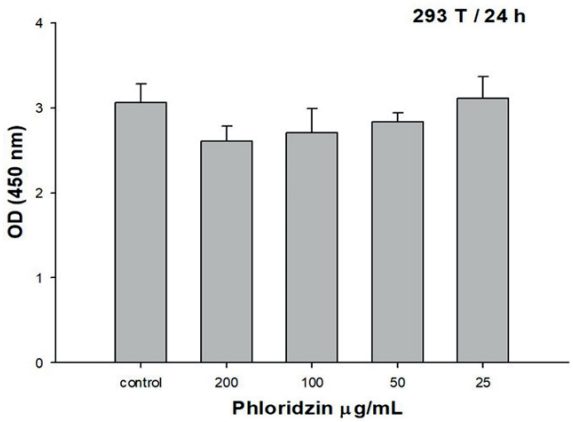

Figure 7. Effect of Phloridzin on cell viability in 293T between 200-25 µg/mL over 24-hour incubation.

DISCUSSION

Cancer can be described as a population of abnormal cells that divide uncontrollably with the ability to invade other tissues. Since the previous half-century, enormous financial and moral efforts have been made to combat cancer, dubbed the world's most serious health problem. The most significant effort in this regard is to bring new treatment approaches against cancer and potential chemotherapeutic agents with high safety to the market. Today, it is clear that developing new therapeutic agents to treat cancer is a top goal for pharmaceutical companies and independent research organisations. All research in this field aims to develop new therapeutic agents that can exhibit selective cytotoxic or antiproliferative effects in cancer cells without causing toxic responses in normal, healthy cells. Hence, developing new agents that are both effective and safe is highly crucial for cancer treatment. We encounter plant-derived extracts or metabolites isolated from plants (5).

National and worldwide epidemiological studies now show that the molecules that enter a person's daily diet and diet are linked to cancer, just as they are with many other diseases. Evidence shows that a diet rich in fruits and vegetables can reduce the risk of common types of cancer; both basic and clinical studies have shown the benefits of certain classes of phytochemicals (24). Flavonoids are precious biological components consumed in significant amounts in our daily diets. Humans are thought to consume roughly 100 mg of flavonoids per day (25, 26). Numerous studies indicate that flavonoids have various biological effects, including antiallergic, anti-inflammatory, antioxidant, antimutagenic, and anticarcinogenic effects (27-29). Flavonoids have stood out among natural agents in recent anticancer studies because they suppress the cell cycle, induce apoptosis, inhibit mitotic spindle formation, and inhibit angiogenesis. Each has different structural characteristics that suggest it could be a potential anticancer agent (30-33).

Besides their nutritional values, apples (Malus spp., Rosaceae) can also prevent and treat various diseases. Several epidemiological and laboratory-based studies have examined the anticancer activity of phenolic chemicals found in apples (34,35). Phloridzin, a dihydrochalcone, is also one of the significant phenolic flavonoid glucosides found in apples (14), and it has antioxidant, anti-inflammatory, and antitumor activities (36,37). Phloridzin also has many pharmacological activities, such as regulating blood sugar levels and blood pressure, protecting the heart, scavenging free oxygen radicals, and antioxidant injuries (13).

In their study, Wang et al. discussed the effect of Phloridzin on the HepG2 human liver cancer cell line and MDAMB 231 human breast adenocarcinoma cell line. They suggested that it has chemotherapeutic effects. Phloridzin showed antagonism on the estradiol-induced proliferation of MCF-7 cells; however, it was observed that it did not significantly affect the proliferation of estrogen-insensitive ER (-) MDA-MB-231 cells. This study demonstrated that Phloridzin exerts a bidirectional modulating function of estrogenic and antiestrogenic activities. It displayed significant effects on the proliferation of estrogen-sensitive estrogen receptor (ER) (+) MCF-7 cells in the absence of estrogen. Wang et al. proved that Phloridzin is distributed in the target organ and plays the role of phytoestrogen (38). According to findings, it was cytotoxically effective at doses

determined statistically on the estrogen-positive cell line MCF-7 but not at any dose on the estrogen negative MDAMB 231 cell line.

Phloridzin inhibited cell growth, invasion, and migration of human liver cancer cells by inducing apoptosis. In addition, Phloridzin has also been shown to have antitumor properties in human leukaemia cells, bladder cancer cells, and human colon cancer cells. The cytotoxic potential of Phloridzin on the cell has been found to inhibit tumour cell growth in vivo, with two inhibitors of glucose transmembrane transport such as Phloridzin (P1) and aglucone phloretin (P2) (39).

For the first time, our study tested Phloridzin flavonoid, a phenolic compound and abundantly in our daily diet, in 200, 100, 50, and 25 µg/mL doses, in a 24-hour incubation period, on five different cancer cell lines. Its cytotoxic effect was tested. While no statistically significant cytotoxic effect was observed on the breast cancer cell lines MDA-MB-231, it was cytotoxic effective on MCF-7. While it has a cytotoxic effect even at low doses in cancer cell lines such as PANC-1, 22RV-1, U87, and A549, it did not show a cytotoxic effect on 293T used for the control group. This indicates that Phloridzin is very important and promising for selective cytotoxicity. Based on this study, we hope that our results will shed light on investigating the mechanism of death of Phloridzin in the indicated cells in more detail and as an alternative treatment to cancer treatments.

Conflict of Interest: The authors have declared that they have no conflict of interest.

Funding: The authors have declared that they have not received any financial support.

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