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DETERMINATION OF THE EFFECT of SAFFRON on MCF-7 and MCF-12a CELL LINES

SAFRAN BİTKİSİNİN MCF-7 ve MCF-12a HÜCRE HATLARI ÜZERİNDEKİ ETKİSİNİN BELİRLENMESİ

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Abstract

Objective: Cancer is considered a major threat to health in the world where response to treatment varies. Recently, there has been great interest in the inhibitory role of herbs and spices in tumor growth inhibition and cancer progression. Saffron, also called 'red gold', is an aromatic medicinal herb. Saffron's medicinal properties, ease of use in the kitchen, and phytochemical profile have led to further investigation of its biological and therapeutic properties. Saffron is rich in carotenoids. Many studies have investigated the effect of saffron and its active ingredients in the prevention and treatment of cancer. Today, the mechanism of action of saffron has not yet been clarified. The aim of this study was to determine the anticancer and antitoxic effects of saffron as a chemo-inhibitory herb in MCF-7 and MCF-12a cell lines, respectively.

Material and Methods: MCF-7 and MCF-12a cells were grown in flasks containing DMEM and DMEM/F-12, respectively. Both groups of cells were treated with saffron concentrations of 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 mL for 24 hours. Then, 20% MTT dye was added to the wells of the aspirated plates and incubated for 3 hours. After stopping the reaction with pure Dimethyl Sulfoxide (DMSO) at the end of the period, the absorbance values of the plates were read spectrophotometrically at a wavelength of 570 nm.

Results: The percent viability values for the MCF-7 cell line were found to be between 50.9 and 66.2%, and the IC50 value was calculated as 92.4 mg/mL. It was observed that the viability values for the MCF12-a cell line ranged between 100.9 and 93.7% after the saffron application.

Conclusion: While saffron showed cytotoxic effect in MCF-7 cell line, it showed anti-cytotoxic effect in MCF-12a cell line. In our study, it was determined that saffron decreased the viability of MCF-7 cancer cells in a dose-dependent manner and did not have a cytotoxic effect on the MCF-12a cell line. More comprehensive controlled clinical studies are needed to demonstrate the use of saffron in combination with other anti-tumor drugs and to improve the artificial synthesis of saffron's active components.

Key Words: Saffron, Cytotoxicity, Breast cancer, Cancer treatment, Cell culture

Özet

Amaç: Kanser, tedaviye yanıtın değişkenlik gösterdiği dünyada sağlık için büyük bir tehdit olarak kabul edilmektedir. Son zamanlarda, şifalı otların ve baharatların tümör büyümesinin inhibisyonu ve kanserin ilerlemesindeki inhibitör rolüne büyük bir ilgi olmuştur. 'Kırmızı altın' olarak da adlandırılan safran, aromatik bir şifalı bitkidir. Safranın tıbbi özellikleri, mutfakta kullanım kolaylığı ve fitokimyasal profili, biyolojik ve terapötik özelliklerinin daha fazla araştırılmasına yol açmıştır. Safran karotenoidler açısından zengindir. Birçok çalışma, safranın ve aktif bileşenlerinin kanserin önlenmesi ve tedavisindeki etkisini araştırmıştır. Günümüzde safranın etki mekanizması henüz netlik kazanmamıştır. Bu çalışmanın amacı, kemo-inhibitör bir bitki olarak safranın antikanser ve antitoksik etkilerini sırasıyla MCF-7 ve MCF-12a hücre hatlarında belirlemektir.

Gereçler ve Yöntemler: MCF-7 ve MCF-12a hücreleri, sırasıyla DMEM ve DMEM/F-12 içeren şişelerde büyütüldü. Her iki hücre grubu da 24 saat boyunca 100, 50, 25, 12.5, 6.25, 3.125 ve 1.56 mL'lik safran konsantrasyonları ile muamele edildi. Daha sonra aspire edilen plakların kuyucuklarına %20 MTT boyası eklendi ve 3 saat inkübe edildi. Süre sonunda saf Dimetil Sülfoksit (DMSO) ile reaksiyon durdurulduktan sonra plakların absorbans değerleri 570 nm dalga boyunda spektrofotometrik olarak okundu.

Bulgular: MCF-7 hücre dizisi için canlılık yüzdesi değerleri %50.9-66.2 arasında bulundu ve IC_{50} değeri 92.4 mg/mL olarak hesaplandı. Safran uygulamasından sonra MCF12-a hücre hattı için canlılık değerlerinin 100.9 -93.7% arasında değiştiği görüldü.

Sonuç: Safranın, MCF-7 hücre hattında sitotoksik etki gösterirken, MCF-12a hücre hattında anti-sitotoksik etki göstermiştir. Çalışmamızda safranın MCF-7 kanser hücrelerinin canlılığını doza bağlı olarak azalttığı ve MCF-12a hücre hattı üzerinde sitotoksik etki göstermediği belirlenmiştir. Safranın diğer anti-tümör ilaçlarla kombinasyon halinde kullanımını göstermek ve safranın aktif bileşenlerinin yapay sentezini geliştirmek için daha kapsamlı kontrollü klinik çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Safran, Sitotoksisite, Meme kanseri, Kanser tedavisi, Hücre kültürü

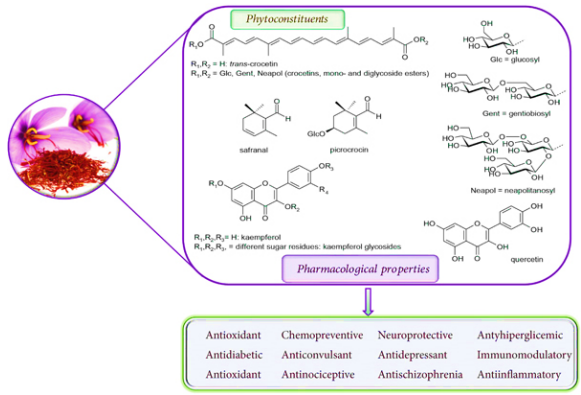
Introduction

Cancer development as a major threat to health in the world, it is a disease that varies in age of onset, invasiveness, and response to treatment.Cancer remains a major leading cause of death worldwide (Milajerdi et al., 2018). Changes in cancer patterns have reduced the role of genetics in observed differences between populations (Abdullaev, 2002). Studies have shown that most cancers can be prevented by lifestyle and environmental changes, including an adequate and balanced diet (Peto, 2001).

Recently, there has been a interest in the inhibitory role of natural products and spices in tumor growth inhibition and cancer progression. Saffron, also called 'red gold', is an aromatic medicinal herb. The name of saffron is derived from the Arabic word "zafaran" meaning yellow. Saffron (*Crocus sativus L.*) is often used in various parts of the world as a spice and as a therapeutic agent for a number of diseases (Wali et al., 2020). Saffron is considered the most expensive traditional spice all over the world. It is estimated that Iran accounts for approximately 76% of the total annual world saffron production (Anand et al., 2008). Saffron has been used in Iranian traditional medicine as a digestive aid, pain reliever, antidepressant, and appetite stimulant (Wang et al., 2022).

Saffron's medicinal virtues, ease of use in the kitchen, and its phytochemical profile have led to further investigation of its biological and therapeutic properties. Saffron is rich in carotenoids. The main products of *Crocus sativus L.* are; crocins and crocetin derived from zeaxanthin, pyrocrocin, and safranal that give it its taste and aroma (Butnariu et al., 2022).

Figure 1. Biological properties of saffron (Abu-Izneid et al., 2022)



The active components of *Crocus sativus L*. have high bioavailability and bioaccessibility and the ability to cross the blood-brain barrier. Numerous preclinical and clinical studies have supported the neuroprotective effects of *Crocus sativus L*. and its bioactive components (safranal, crocin and picrocrocin). The active compounds of *Crocus sativus L*. may be a useful medicinal food ingredient in the creation of medicines for certain diseases (Abu-Izneid et al., 2022).

Many studies have investigated the effect of *Crocus sativus L*., it is active ingredients in the prevention and treatment of cancer. Today, the mechanism of action of *Crocus sativus L*. has not yet been clarified. The aim of this study was to determine the anticancer and antitoxic effects of *Crocus sativus L*. as a chemo-inhibitory herb in MCF-7 and MCF-12a cell lines, respectively.

2. Material and Methods

2.1. Test Item

Safran, Saharkhiz Saffron Co. supplied by. 1 mg/mL main stock was prepared by dissolving the *Crocus sativus L.* plant in distilled Dimethyl sulfoxide in the experimental study. Other concentrations (100; 50; 25; 12.5; 6.25; 3.125; 1.56 mg/mL) were prepared by serial dilutions of the 1 mg/mL master stock concentration.

2.2. Cell Culture

In cell culture studies, necessary media and an appropriate environment were provided for the cells to live and reproduce in vitro. The medium requirement differs according to the type of cells and their adaptability. Our study used three different media (DMEM and DMEM/F-12) for two different cell lines (MCF-7 and MCF-12a). The media of the cells were kept in an incubator at 5% CO_2 and 37°C, 95% humidity were changed twice a week and the development of the cells was monitored.

2.3. MTT Analysis

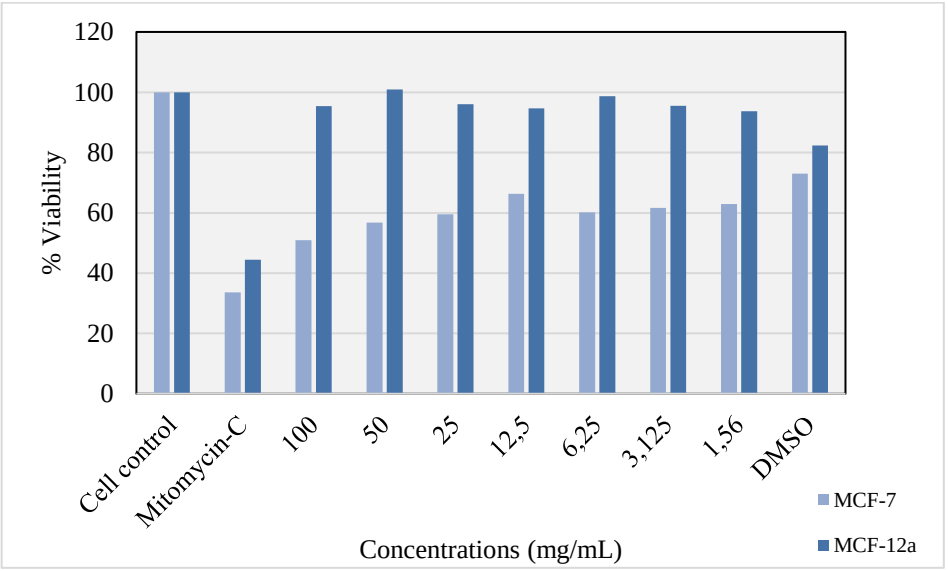
The purpose of using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) method. The cells were seeded onto the plates with the help of a multi-pipe so that the calculated amount of cells was poured into each well of the 96-well plates. The seeded cells were kept in the incubator for 24 hours to adhere to the plate surface. Other concentrations to be studied (100; 50; 25; 12.5; 6.25; 3.125; 1.56 mg/mL) were prepared with serial dilutions of the master stock concentration prepared with *Crocus sativus L.* used in the experimental study, and *Crocus sativus L.* at the concentrations prepared for each cell line was added to the plate in

triplicate. Negative control (cell control), positive control (mitomycin-C) and 1/1000 DMSO concentrations were added to the plate in triplicate and left in the incubator for 24 hours. Since MTT dye is a light-affected dye, 5 mg was weighed for 1 plate in the dark, 1 mL of PBS (phosphate buffered saline) was added to it and 8 mL of medium was added and dissolved by vortexing. The prepared MTT solution was inoculated on the plates and the plates covered with aluminum foil were kept in the incubator for 2-4 hours. The MTT solution was aspirated and 100 µL of DMSO (100%) was added to each well to stop the reaction. After the plate was kept in the dark for 10 minutes, absorbance values were read spectrophotometrically at a wavelength of 570 nm. The effect of the MTT method on cell density in working cell lines was determined with the concentrations applied with the help of the Microsoft Excel program.

3. Results and Discussion

The MCF-7 cancer cell line; it showed the best effect on the cell viability of the *Crocus sativus L.* plant at 100 mg/mL concentration. The % viability activities in the MCF-7 cancer cell line were determined between 50.9 and 66.2%. Therefore, it is thought to have antiproliferative activity in the MCF-7 cancer cell line. The % viability activities in the MCF-12a cell line were determined between 100.9 and 93.7%. Comparison of percent viability values of two cell lines is shown in figure 2.

Figure 2. Percent % viability values of MCF-7 and MCF-12a cell lines



The effect of the *Crocus sativus L.* on cell density was read with a spectrophotometer using the MTT method, the percent viability curve was determined with the help of the Microsoft Excel program and the 50% inhibitory concentration value (IC_{50}) was calculated with a bar graph and a logarithmic slope line was drawn. The 50% inhibitory concentration (IC_{50}) value from the logarithmic slope line of the MCF-7 cell line was determined as 92.4 mg/mL.

In a study conducted in 2018, to elucidate the antiproliferative potential of *Crocus sativus L.* extract and its main components, crocin and safranal, were found in five different cell lines; The alveolar lung epithelial cancer cell line [A549], breast epithelial cancer cell line [T47D], colon colorectal cell line [HCT-116], and prostrate cancerous cell line [PC3] and nonmalignant cell line [L929] were investigated. The content of crocin and safranal in saffron extract was measured.
All cells were incubated with different concentrations of CSE, crocin, and safranal for 48 hours. In a concentration-dependent manner, both safranal and crocin reduced cell proliferation in all malignant cell lines. IC_{50} values ranged from 0.32 to 0.42 mM for safranal, 0.31 to 0.92 mM for crocin, and 0.58 to 0.98 mg/mL for *Crocus sativus L.* extract (Bukhari et al., 2018). Based on the findings of this study, although the cell lines we used in our experimental study are different, saffron has an effect on cell lines, suggesting that saffron can be used as a promising chemotherapeutic agent in the treatment of cancer in the future.

Tavakkol-Afshari et al. evaluated the cytotoxic effect of *Crocus sativus L.*extract in HepG2 and HeLa cell lines and investigated the role of apoptosis and ROS. *Crocus sativus L.* can decrease cell viability in malignant cells in a concentration- and time-dependent manner. The IC_{50} values against HeLa and HepG2 cell lines were 800 and 950 µg/mL after 48 hours, respectively. It can be concluded that *Crocus sativus L.* may cause cell death in HeLa and HepG2 cells, in which apoptosis or apoptosis or apoptosis plays an important role (Tavakkol-Afshari et al., 2008). Although IC_{50} values are higher than our values due to the concentration in this experimental study, the result supports our study of the antitoxic effect of saffron.

Parizadeh et al. showed an increase in the cytotoxic effect with the addition of the extract at concentrations of 0, 200, 400 and 800 µg/mL in the MTT test results of their study with saffron. However, the NO concentration decreased significantly after 6, 12, 18, 24, 48 and 72 hours of incubation, respectively. An IC_{50} of 400 µg/mL was obtained for HepG2 cells (Parizadeh et al., 2011).

The 2021 experimental study aimed to evaluate the effects of crocin administration on anxiety, depression, and chemotherapy toxicity profile during doxorubicin-based chemotherapy of breast cancer. 72 patients with non-metastatic Her2/neu positive or triple negative breast cancer were randomized to receive 30 mg/day crocin or placebo during chemotherapy. The results show that the use of crocin during chemotherapy improves anxiety and depression in patients with breast cancer. In addition, while leukopenia increased in the crocin group, hypersensitivity reaction and neurological disorders decreased (Salek et al., 2021). This experimental study shows that saffron supports chemotherapy when used as a spice and predicts that saffron can be used together with chemotherapy drugs in the future.

4. Conclusion

Cancer is recognized as a major threat to health in the world, where response to treatment varies. Natural plants have long been used to prevent and treat many diseases, including cancer. It has been encouraging that *Crocus sativus L.* and its components can affect carcinogenesis and that it has been studied as one of the cancer chemopreventive agents. While *Crocus sativus L.* and its active natural compounds are promising anticancer candidates, saffron extract needs extensive therapeutic evaluation at higher concentrations.

Conflicts of interest

Conflicts of interest The authors declare that there are no potential conflicts of interest relevant to this article.

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