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Original article

Saffron induces Apoptosis in Ovarian Cancer cell via MAPK and AKT/mTOR Pathways

Ayşe Güler Okyay[1], Halil Mahir Kaplan[2], Hasan Asil[3], Ergin Singirik[4]

[1] Department of Obstetrics and Gynecology, Tayfur Ata Sökmen Medical Faculty, Mustafa Kemal University, Hatay/Turkey
[2] Pharmacology Department, Çukurova University, Adana/Turkey
[3] Medicinal and Aromatic Plants, Altınözü Vocational School of Agricultural Sciences, Mustafa Kemal University, Hatay/Turkey
[4] Pharmacology Department, Çukurova University, Adana/Turkey

Abstract. *Study Objectives:* Saffron and its main components have been shown to have anti-tumor and anti-cancer effects in animal studies and human cancer cell cultures. This study aimed to investigate the anti-cancer effects of saffron on human ovarian cancer cells. *Methods:* Powder of saffron was applied to the HO–8910 cell lines. Cell viability was determined. ELISA test is used to examine the activity of caspase-3 and expression of AIF, gadd153, grp78, caspase 3, bax, bcl-2, wee 1, which are apoptotic pathway's mediators. Active ERK (p-ERK), active JNK (p-JNK) active AKT (p-AKT), and active mTOR (p-mTOR) were also analyzed by ELISA. *Results:* Saffron treatment reduced the viability of ovarian cancer cells. Saffron treatment increased activity of caspase 3 and expression of bax, wee 1, gadd153, grp78, and AIF and decreased bcl-2 which is anti-apoptotic protein. Saffron also decreased the activity of p-ERK, p-JNK, p-AKT, and p-mTOR in ovarian cancer cells. *Conclusion:* This study revealed that saffron has a beneficial effect on cancer treatment. Saffron may show a synergistic effect with various chemotherapeutics while directing the cancer cell to death. Crocetin, one of its active components, has shown a synergistic anti-cancer effect combined with cisplatin. Saffron induced apoptosis via ER stress, AKT/mTOR, and MAPK pathways in the ovarian cancer cell line.

Keywords: Saffron, ovarian cancer, apoptosis, MAPK, AKT/mTOR

Introduction

Although great improvement in the medical and pharmaceutical field, cancer has remained as an incurable disease. This led the researchers to seek alternative treatments. Traditional medicine knowledge has been revised all over the world and therapeutic effects began to be studied in depth. Crocus Sativus is the raw material of saffron, the most expensive spice in the world, and has been used in traditional medicine for centuries.

Saffron has been used in traditional medicine as an appetizer, stomach acid regulator, tranquilizer, expectorant, aphrodisiac, abortifacient, for treatment of liver diseases, gas, and spasm relief, tooth and gum ailments, insomnia, cough, bronchitis, colds, fever, heart diseases, and cancer (1-3). Modern biomedical findings showed that saffron and its components may be useful in the treatment of neuro-degenerative diseases and resultant memory problems, ischemic neuropathy, age-related macular degeneration, coronary artery disease, blood pressure abnormalities, acute and chronic inflammatory diseases, mild-moderate depression, seizure, and Parkinson's disease (4,5). In addition, anti-oxidant, anti-mutagenic, anti-genotoxic and tumoricidal effects have been found saffron to have (6,7).

As a result of the chemical analysis of saffron flowers (Figure 1), it has been determined that it has more than 150 components. It is known that the strongest

components of saffron are crocin, crocetin, and safranal (8). In animal studies and human cancer cell cultures, saffron and its main components have been shown to have anti-tumor and anti-cancer effects, and the possible mechanisms of these effects have been investigated and are under investigation (1,9,10).

The anti-carcinogenic effect of saffron was first reported in the 1990s and studies on this issue have been accelerating since then. Recent studies on saffron are encouraging and saffron and its' derivatives have been found to affect carcinogenesis in many in vivo and in vitro models. In particular, crocin and crocetin components have been shown to have significant anti-cancer activity on breast, lung, pancreas, and leukemia cells (6,7,9). The effects of saffron on gynecological cancers such as cervical and ovarian cancer cell cultures were investigated and crocetin was found to show anti-proliferative effects depending on concentration. Crocetin inhibits DNA and RNA synthesis in cervical cancer cells and accelerates apoptosis in a time-dependent manner (6,11)

Apoptosis has an important function in some cancers through both developments of cancer itself and resistance gain against chemotherapeutic agents. Apoptosis is programmed cell death that functions in

Figure 1. Saffron flower

the development of tissue, removing damaged tissue and reshaping of tissue, homeostasis, and aging (9,12). Recent research on the stimulation or inhibition of apoptosis has brought a new perspective to cancer treatment. While stimulated apoptosis causes organ failure, inhibition of apoptosis causes cancer and hyperplasia. Apoptosis disorders are important in developmental, autoimmune, and neuro-degenerative diseases and cancer development. It is reported in literature that anti-apoptotic proteins are responsible for resistance to chemotherapeutics. It has been reported that anti-apoptotic bcl-2 proteins are activated in many cancers (13-15).

The study aimed to investigate the anti-cancer effects of saffron on ovarian cancer cells. For this pupose; we planned to analyze the apoptosis mediators and mitosis factors at the molecular level. In recent studies, saffron has been shown to alter the activities and expression of caspase-3, BAX, and bcl-2 protein, mediators of apoptosis. Caspase 3 and BAX are pro-apoptotic mediators. Bcl-2 is an anti-apoptotic mediator (15,16). In the study, evaluation of the expression and activities of those mediators, and also evaluation of the activities of mitosis factors AIF, Wee1, Gadd53, and Grp-78 were aimed. Ovarian cancer is the ninth most common women cancer and with a 5-year survival rate of 46%, it is the most fatal gynecological cancer probably due to delayed diagnosis and inadequate treatment (17).

Ovarian cancer is generally not inherited. However, up to 24% of cases may be related to a genetic mutation, even if the family history is negative for ovarian or breast cancer. There are epithelial, stromal and germ cell ovarian cancer types depending on the type of cell it originated from. Ovarian cancer and many other conditions unrelated to cancer may cause similar symptoms. Unfortunately, in the early stages, ovarian cancer may not cause noticeable symptoms. Some symptoms are very vague and many other common diseases such as digestive system problems also can cause them. So, women may not pay attention to those symptoms. Treatment of ovarian cancer usually includes a combination of surgical intervention and chemotherapy (17,18).

Saffron has been studied in many cancer cells. However, the effects of this substance on caspase 3, bax, bcl2, wee 1, gadd 153, grp 78, and AIF proteins on human ovarian cancer cells were not studied. Revealing the expression and activities of these mediators one by one will increase the original value of our study.

Material and Method

This study was supported financially by the Coordination of Scientific Research Projects (SRP) of Hatay Mustafa Kemal University.

10 grams of powder of saffron mixed with 80% (v/v) aqueous ethanol and the mixture was macerated for five days at 4°C. The resulting mixture was then filtered dried under reduced pressure in a rotary evaporator at 40°C to give water and ethanol crude extracts.

RIPA buffer, fetal bovine serum (FBS), phosphate-buffered saline (PBS), NaCl, TritonX-100, Ethylene glycol tetraacetic acid (EGTA), dithiothreitol, NaF, Tris–HCl, Na_3VO_4 were obtained from Sigma-Aldrich, Inc. RPMI 1640 medium and HO-8910 cell lines were obtained from ATCC Inc. ELISA kits of caspase-3, AIF, gadd153, grp78, caspase 3, bax, bcl2, wee 1, gadd153 were purchased from Shanghai Sunred Biological Technology Co., Ltd. ERK (p-ERK), JNK (p-JNK) akt (p-AKT) and mTOR-(p-mTOR) ELISA kit were purchased from Mybiosource Inc. In addition, the Bradford dye reagent was purchased from Bio-Rad Laboratories, Inc.

Cell Culture

5 mg/mL dose of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; sigma) were prepared by dissolving in filtered PBS. Cells were plated at a density of 104 cells/cm2 in 96-well plates in a final volume of 180 μL of medium and were incubated overnight. The cells were then treated once with a dose of 1,2,4 and 8 mg/ml of Saffron extract and were observed after 48h. After completion of the treatment with Saffron, MTT was added to each well at a 1/10 volume for 3 h at 37°C. The

supernatants were carefully aspirated, 100 μL of dimethyl sulfoxide was added to each well, and the plates were agitated to dissolve the crystal product. The absorbance of plates was measured at 570 nm Quantitative Analysis.

Cell Homogenization

Cells (5 × 104 cells/cm2) were exposed to 8mg/ml saffron for 48 h. They were then washed in PBS and lysed in RIPA buffer (150 mmol/L NaCl 0.5%, TritonX-100, 20 mmol/L EGTA, 1 mmol/L dithiothreitol, 25 mmol/L NaF, 50 mmol/L Tris–HCl [pH 7.4], 1 mmol/L Na_3VO_4) for 15 min on ice followed by centrifugation at 15000 rpm for 20 min. and supernatants are taken and pellets are discarded.

Total protein determination

Bradford method is used to determining of the total protein in homogenized tissues. Protein determination (μg/μl) is done according to the standard curve drawn in Prism software.

ELISA (Enzyme Linked Immunosorbent Assay) Tests

ELISA test is used to examine the activity of caspase 3 and expression of AIF, gadd153, grp78, caspase 3, bax, bcl2, wee 1, which are apoptotic pathway's mediators. Active ERK (p-ERK), active JNK (p-JNK) active AKT (p-AKT) and active mTOR (p-mTOR) were also analyzed by ELISA.

Statistical Analysis

The Independent Sample t test (Student's t test) was used for the comparison of parameters from Saffron treated and control groups. Data are presented as means ± SEM. Statistical analysis of differences with $p < 0.05$ was taken as the indicator of significance.

Results

Saffron treatment reduced the viability of ovarian cancer cells (Figure 2). Saffron treatment increased the activity of caspase3 and expression of bax (Figure 3), wee 1, gadd153, grp78, and AIF (Table I) and decreased bcl2 (Figure 4) which is antia-poptotic protein. Saffron also decreased activity of p-erk, p-jnk, p-akt, and p-mtor (Figure 5) in ovarian cancer cells.

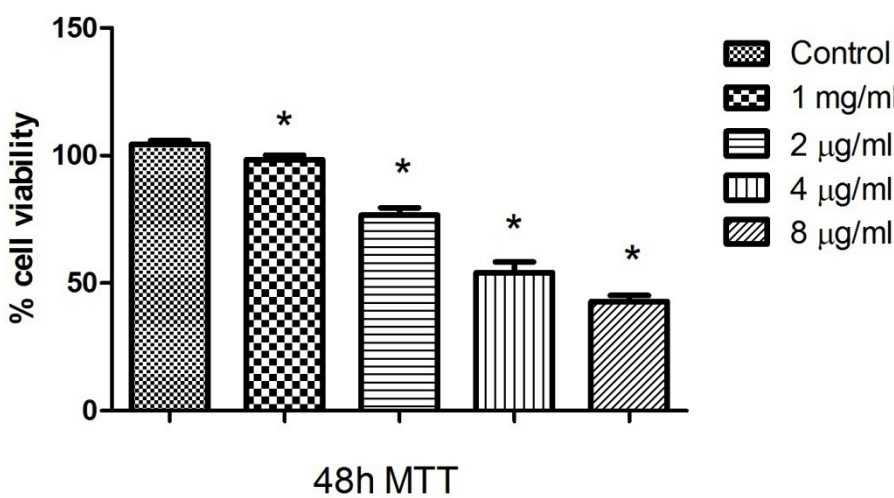

Figure 2. Saffron treatment reduced viability of ovarian cancer cell.

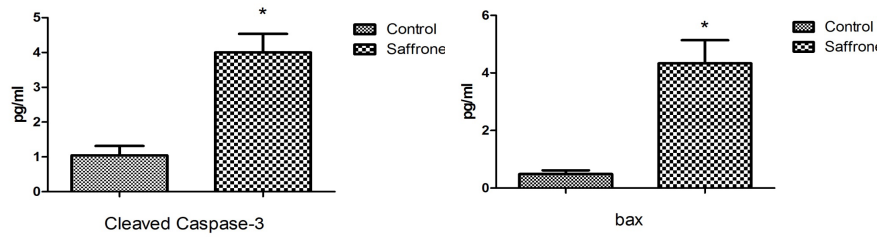

Figure 3. Saffron treatment increased the activity of caspase 3 and expression of bax

Table 1. Effects of saffron treatment on expression of wee 1, gadd153, grp78, and AIF

	Control	**Saffron**
wee 1	0.32±0.02pg/ml	0.68±0.01pg/ml*
AIF	1.1±0.02pg/ml	1.6±0.09pg/ml*
gadd153	0.36±0.03pg/ml	2.1±0.3pg/ml*
grp78	0.66±0.02pg/ml	1.25±0.13pg/ml*

** : For control p < 0.05*

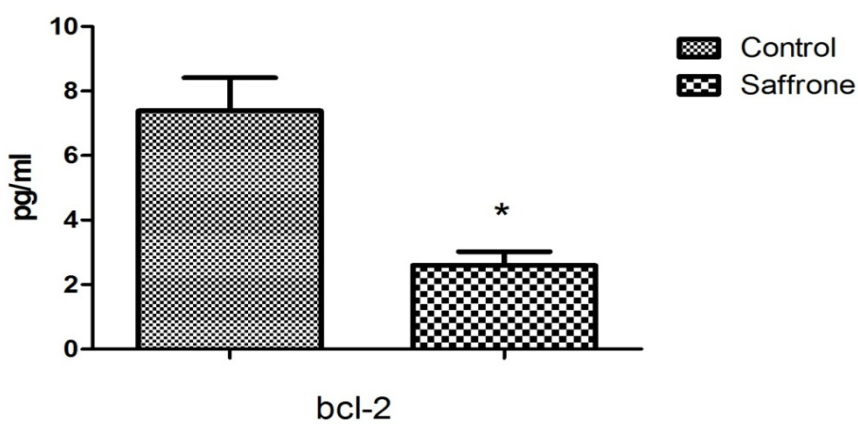

Figure 4. Saffron treatment decreased bcl2.

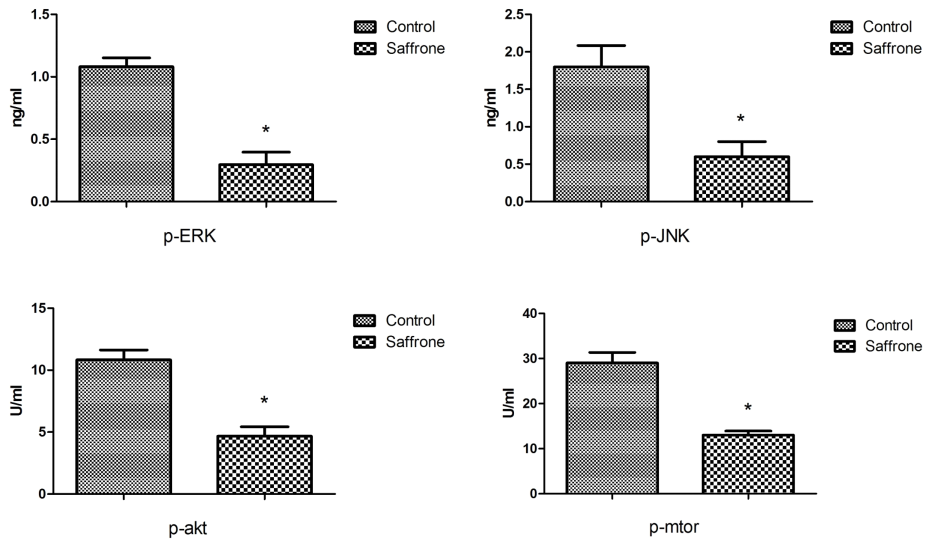

Figure 5. Decreased activity of p-ERK, p-JNK, p-AKT and p-mTOR in ovarian cancer cells.

Discussion

The saffron is extensively used in medicine to treat multiple diseases such as vascular diseases, inflammatory and autoimmune diseases (8,12). Previous studies showed saffron has the potentials for cancer prevention and therapy. In vitro studies have shown that saffron is effective in many types of cancer (3,19,20). In this study, we aimed to reveal the molecular mechanism of anticancer activity of saffron by analyzing ER stress, apoptotic, AKT/mTOR, and MAPK pathways.

Saffron induced ER stress by increasing IRE1, ATF6, Gadd153, and grp78 which is endoplasmic reticulum (ER) stress mediators. ER stress, which restores homeostasis or activates cell death, is activated by a variety of factors and ER stress mediators. Lots of studies have shown the correlation between ER stress and cancer, and particularly the involvement of ER stress mediators. ER stress mediators modulate the paradoxical microenvironment of cancer and it is also one of the resistance mechanisms against cancer therapy (21).

Saffron treatment also inhibited mitogen-activated protein kinase (MAPK) pathways. MAPK pathways link extracellular signals to growth, proliferation, differentiation, migration, and apoptosis. Drugs targeting this pathway are currently being developed (22,23). Furthermore, AKT/mTOR pathway also inhibited by saffron. This pathway has an important role in regulation of the cell cycle. Inhibitors of this pathway are being tested in the clinic (24).

The goal of all chemotherapeutics is to lead the cancer cell to death. Saffron-induced apoptosis has been shown in this study. Apoptosis is also known as cell death. The interaction of saffron with all those mentioned pathways was important, but it was more important to induce apoptosis itself. This study showed the molecular mechanism of the anti-cancer activity of saffron. There were lots of studies that showed anticancer activity of saffron, especially with its active ingredients (19,25).

Crocin is a main active component of saffron and inhibits cancer cell proliferation and induces cell apoptosis by inhibition of enzymes which induces nucleic

acid synthesis (9, 19). Crocin has also shown protective roles for the prevention of early liver cancer (25). Moreover, crocin inhibits human liver cancer cell proliferation and induces apoptosis by suppressing the activity of AKT/mTOR pathway (24).

Conclusion

In conclusion; this study revealed that saffron has a beneficial effect on cancer treatment. Saffron may show a synergistic effect with various chemotherapeutics while directing the cancer cell to death. Crocetin one of its active components has a synergistic anticancer effect combined with cisplatin. Saffron induced apoptosis via ER stress, AKT/mTOR, and MAPK pathways in the ovarian cancer cell line.

Conflicts of Interest

The authors declare that there is no conflict of interest in this manuscript.

References

1. Samarghandian S., Borji A. Anticarcinogenic effect of saffron (Crocus sativus L.) and its ingredients. Pharmacognosy Research 2014; 6 (2): 99–107.
2. Kianbakht S, Mozaffari K. Effects of saffron and its Active Constituents, Crocin and Safranal, on Prevention of Indomethacin Induced Gastric Ulcers in Diabetic and Nondiabetic Rats. J Med Plants 2009;8:30–8.
3. Hoshyar, R. and H. Mollaei, A comprehensive review on anticancer mechanisms of the main carotenoid of saffron, crocin. Journal of Pharmacy and Pharmacology, 2017. 69(11): p. 1419–1427.
4. Shafiee, M., et al., Saffron in the treatment of depression, anxiety and other mental disorders: Current evidence and potential mechanisms of action. Journal of Affective Disorders, 2018. 227: 330–337.
5. Khazdair, M.R., et al., The effects of Crocus sativus (saffron) and its constituents on nervous system: A review. Avicenna J Phytomed, 2015. 5(5): p. 376–91.
6. Abdullaev FI, Espinosa-Aguirre JJ. Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. Cancer Detect Prev 2004;28:426–32.
7. Yao, C., et al., Crocin induces autophagic apoptosis in hepatocellular carcinoma by inhibiting Akt/mTOR activity. Onco Targets Ther, 2018. 11: p. 2017–2028.
8. Asil, H., (2018). Safran'ın (Crocus sativus L.) Tıbbi ve Farmakolojik Özellikleri, 1. International Mersin Symposium, Conference Proceedings 2, 145–155.
9. Hoshyar, R., S.Z. Bathaie, and M. Sadeghizadeh, Crocin Triggers the Apoptosis through Increasing the Bax/Bcl-2 Ratio and Caspase Activation in Human Gastric Adenocarcinoma, AGS, Cells. DNA and Cell Biology, 2013. 32(2): p. 50–57.
10. Chen, S., et al., Crocin inhibits cell proliferation and enhances cisplatin and pemetrexed chemosensitivity in lung cancer cells. Transl Lung Cancer Res, 2015. 4(6): p. 775–83.
11. Samarghandian S, Borji A, Farahmand SK, Afshari R, Davoodi S. Crocus sativus L. (Saffron) Stigma Aqueous Extract Induces Apoptosis in Alveolar Human Lung Cancer Cells through Caspase-Dependent Pathways Activation. Biomed Res Int 2013; 2013:1–12.
12. Çıtak EÇ. 2000. «Apoptosis and cancer». Turkiye Klinikleri J Pediatr, 9(3),183–91.
13. Yazıcı P, Alizadehshargh S, Akdoğan GG. 2009." Apopitoz: Düzenleyici Moleküller, hastalıklarla İlişkisi ve Apopitozu Saptama Yöntemleri." Turkiye Klinikleri J Med Sci, 29(6),1677–86.
14. Call JA, Eckhardt SG, Camidge DR. "Targeted manipulation of apoptosis in cancer treatment. Lancet Oncol," 2008; 9(10):1002.
15. Heiser D, Labi V, Erlacher M, Villunger A. "The Bcl-2 protein family and its role in the development of neoplastic disease." ExpGerontol, 2004; 39(8): 1125–35.
16. Mousavi S.H., Tavakkol-Afshari J., Brook A., Jafari-Anarkooli I. Role of caspases and Bax protein in saffron-induced apoptosis in MCF-7 cells. Food and Chemical Toxicology 2009;47:1909–1913.
17. Berek, J.S., Natarajan S., Ovarian and Fallopian Tube Cancer, Berek& Novak's Gynecology, Lippincott William &Wilkins 2007. 14th edition, p1457–1548.
18. Kathawala R.J., Kudelka A., Rigas B. The Chemoprevention of Ovarian Cancer: the Need and the Options. Current Pharmacol Rep. 2018;4(3): 250-260. DOI: 10.1007/s40495-018-0133–6.
19. Hoshyar, R. and H. Mollaei, A comprehensive review on anticancer mechanisms of main carotenoid of saffron, crocin. Journal of Pharmacy and Pharmacology, 2017. 69(11): 1419–1427.
20. Moradzadeh, M., et al., Anti-tumor effects of crocetin and related molecular targets. Journal of Cellular Physiology, 2018. 233(3): p. 2170–2182.
21. Wang, W.A., J. Groenendyk, and M. Michalak, Endoplasmic reticulum stress associated responses in cancer. Biochimica Et Biophysica Acta-Molecular Cell Research, 2014. 1843(10): p. 2143–2149.
22. Dhillon, A.S., et al., MAP kinase signalling pathways in cancer. Oncogene, 2007. 26(22): p. 3279–3290.

23. Germann, U.A., et al., Targeting the MAPK Signaling Pathway in Cancer: Promising Preclinical Activity with the Novel Selective ERK1/2 Inhibitor BVD-523 (Ulixertinib). Molecular Cancer Therapeutics, 2017. 16(11): p. 2351–2363.
24. Aziz, A.U.R., et al., PIM Kinases and Their Relevance to the PI3K/AKT/mTOR Pathway in the Regulation of Ovarian Cancer. Biomolecules, 2018. 8(1).
25. Amin, A., et al., Saffron: A Potential Candidate for a Novel Anticancer Drug against Hepatocellular Carcinoma. Hepatology, 2011. 54(3): p. 857–867.

Correspondence:
Ayşe Güler Okyay
Department of Obstetrics and Gynecology, Tayfur Ata Sökmen Medical Faculty, Mustafa Kemal University, Hatay/Turkey
E-mail: doctorayseguler@yahoo.com.tr