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The effect of crocin on the proliferation, inflammation, drug synergism, and angiogenesis in breast cancer

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ABSTRACT: Radiation, chemotherapy, and surgery are common treatments for breast cancer. These approaches, however, could only improve the odds of surviving. Using herbal medicines like crocin is now necessary to improve cancer treatment, particularly for breast cancer. According to research, crocin inhibits the growth of cancerous cells while having no effect on the development of healthy cells. By eating this substance, the side effects of cancer chemotherapy are also reduced. Thus, this review delves into the effects of crocin on breast cancer, encompassinganti-angiogenesis, drug synergism, proliferation, and inflammation. Crocin decreases the expression of CD34 in tumor tissues, leading to anti-angiogenesis. Furthermore, the anti-angiogenic effect of crocin can hinder vascular endothelial cell proliferation, migration, and tubule formation in vitro, as well as tumor cell proliferation and reduction in microvascular density in vivo. Also, crocin increases the sensitivity of PTX in breast cancer cell lines.

Keywords: Crocin; Breast cancer; Proliferation; Inflammation; Drug synergism; Anti-angiogenesis

1. Introduction

Common treatments for breast cancer include radiotherapy, chemotherapy, and surgery. But these techniques could only increase the chances of survival. These days, improving the treatment of cancer, especially breast cancer, requires the use of herbal medications like crocin (Vali et al., 2015, Burguin et al., 2021, Hortobagyi, 1992, Trayes and Cokenakes, 2021, Tong et al., 2018, Carlson et al., 2003, Rameshrad et al., 2018). The primary chemical component of saffron, crocin, exhibits anti-tumor activity against a variety of cancer form. Crocin has the potential to suppress growth and induce apoptosis in a wide range of cancer cells. It has demonstrated anti-hyperlipidemia, anti-oxidative, cardioprotective, anti-atherosclerotic, liver protective, and neuroprotective properties in both in vitro and in vivo studies (Jia et al., 2024, Hoshyar and Mollaei, 2017, Bhia et al., 2021, Ruba et al., 2017, Bao et al., 2023, Mollaei et al., 2017, Bakshi et al., 2022, Chen

et al., 2015). Research has shown that crocin stops the growth of cancer cells while having no effect on the growth of healthy cells. These effects have been studied on breast, leukemia, cervical, bladder and colorectal cancer cells (Veisi et al., 2020, Lu et al., 2015, Amin et al., 2016, Zhang et al., 2018, Razavi et al., 2020). The adverse effects of cancer chemotherapy are also lessened by consuming this substance (Hajizadeh and Ghorbian, 2022, Soeda et al., 2007).

This review thus explores the effects of crocin on breast cancer, including proliferation, inflammation, drug synergism, and anti-angiogenesis.

2. Anti-angiogenesis effect

Prolonged exposure to crocin has been shown to have effects on mice, human umbilical vein endothelial cells (HUVECs), and the breast cancer cell MDA-MB-231. The morphology and rate of proliferation of MDA-MB-231 and HUVECs were significantly impacted by crocin. Additionally, it caused dose-dependent cell cycle arrest and apoptosis in these cells at the G2/M phase, as shown in **figure 1**. This demonstrates that HUVEC inhibition is induced by crocin. Additionally, following crocin treatment, the expression of CD34 in tumor tissues decreased (as an anti-angiogenesis effect) (Chen et al., 2019). Additionally, crocin (5 mg/ml) was administered to the mice seven times in a naked mouse model of the breast MDA-MB-231 cell. Following crocin treatment of MDA-MB-231 subcutaneous xenotransplanted tumors, CD34 and Ki-67 expression were measured using immunohistochemistry. The MTT assay demonstrated that crocin considerably inhibited MDA-MB-231 cell proliferation ($P<0.05$), with an IC50 of 5 points0 mg/ml after 48 h. Over the course of 24 hours, crocin had a modest inhibitory effect on HUVECs that was not dose-dependent. The IC50 for 48 hours was 5.97 mg/ml, but the effect was completely opposite at 48 and 72 hours. In MDA-MB-231 cells in the G2/M phase, crocin dose-dependently ($P < 0.05$) created apoptosis and cell cycle arrest. It might also, in a dose-dependent way, suppress HUVEC migration ($P < 0.05$) and tube creation ($P < 0.05$). Ki-67 and CD34 expressions were 26.00±2.65 and 14.67±4.16 ($P < 0.05$), 502.67±88.48, and 262.67±75.08 ($P < 0.05$), respectively, in the blank group and the 5 mg/ml crocin group. These findings demonstrated that the crocin effect of anti-angiogenesis can impede vascular endothelial cell proliferation, migration, and tubule creation in vitro, as well as tumor cell proliferation and microvascular density reduction in vivo (Chen ShuangShuang et al., 2016).

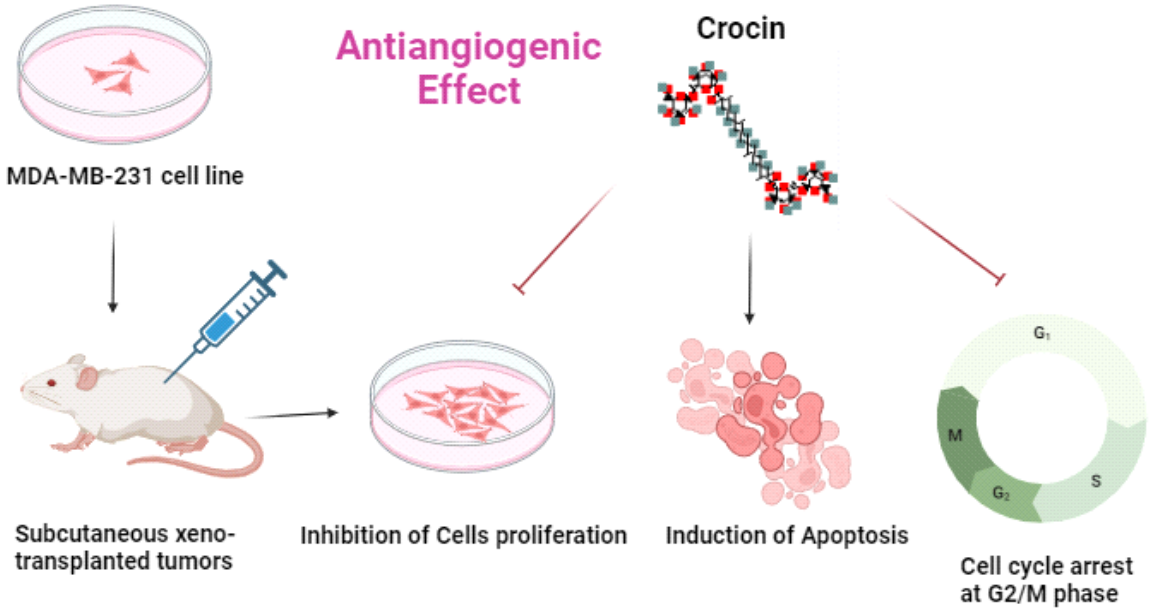

Figure 1: Antiangiogenic effect of crocin. It showing effect mediating through interfering multiple pathway.

3. Effect on the proliferation, inflammation, Cell Cycle and apoptosis

The impact of crocin on the proliferation and inflammation of breast cancer cells was investigated through western analysis of nuclear factor kappa B (NF-κB) and protein kinase C theta (PRKCQ). The rates of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were measured by RT-qPCR and ELISA to assess inflammation. The findings revealed that crocin effectively hindered NF-κB activation, leading to a reduction in cell viability and proliferation in breast cancer cells. The administration of Crocin resulted in a notable decrease in the levels of IL-1β and TNF-α, indicating that Crocin effectively suppressed inflammation in these cells. Moreover, the PharmMapper database identified PRKCQ as a potential goal of Crocin. By reducing the expression of PRKCQ, Crocin treatment successfully hindered the stimulation of NF-κB in BC cells. Consequently, Crocin exhibited its inhibitory effects on NF-κB-mediated proliferation and inflammation in breast cancer cells via the downregulation of the expression of PRKCQ (Xu et al., 2022).

To test the effect of crocin on cell viability and apoptosis, BT-474, a recognized HER2+ breast cancer cell line, was employed. The findings demonstrated that the application of crocin reduces the viability of BT-474 cells and causes both early and late apoptosis in these entities. Inducing expression of caspase-9 and cleavage was the mechanism by which crocin acted. It's also been demonstrated that in these cells, crocin stimulates XBP1 gene splicing. Apoptosis or an adaptive response may ensue from the unfolded protein response (UPR), which is prompted by endoplasmic reticulum (ER) stress. Molecular chaperone expression is enhanced by UPR activation, which also inhibits protein translation and increases transcription factors. More protein folding capacity in the ER and a reduction in protein load are the overall effects of this process. Cells may go through the apoptotic phase if the ER stress is sustained. In particular, the crosstalk between UPR and apoptosis in cancer cells may contribute to the anticancer effects of crocin through UPR stimulation (Faridi et al., 2019, Cawley et al., 2011).

According to an analysis of the effects of paclitaxel, crocin, and radiation on the survival rate of MCF-7 cells, administering almost 2.5 mg/mL of crocin and 0.01 μmol/mL of paclitaxel together for 48 hours could result in an IC50 for the MCF-7 cell line. Also, apoptosis in MCF-7 cell lines could increase from 21% (associated with by 2 Gy gamma alone) to 46% when crocin and 2 Gy gamma radiation are used in combination therapy. Thus, paclitaxel and crocin, gamma radiation, crocin had strong synergistic influence on MCF-7 to create considerable apoptosis, as depicted in **figure 2** (Vali et al., 2015). The impact of *C. sativus* extract (CSE) and crocin on the induction of caspase-mediated cell death in MCF-7 cells, as well as their toxicity profiling and immune stimulatory effect in preclinical studies, demonstrated a gradual decrease in the anti-apoptotic protein Bcl-2 over time. At the same time, there was a concurrent increase in the expression of Bax in MCF-7 cells treated with CSE and crocin. After 24 hours, the administration of CSE and crocin resulted in the downregulation of caspase 8 and 9, as well as the cleavage of caspase 3. Histological studies conducted to assess the in vivo toxicity profile showed no discernible histopathologic variances in the spleen, kidney, liver, heart, and lungs between the groups treated with CSE and those untreated. Peritoneal macrophages exhibited a notable increase in ex vivo yeast phagocytosis in response to crocin treatment, which was both dose and time - dependent. At the molecular level, CSE and crocin were found to induce pro-apoptotic and caspase-mediated cell death in MCF-7 cells, together with significant DNA destruction (Bakshi et al., 2016). The effect of crocin toxicity on MCF-7 cells, specifically proliferation and apoptosis altogether analysis of the expression of an apoptosis-related gene (PTEN) and genes related to the Akt pathway revealed that this herbal drug hindered the proliferation of mentioned cells and triggered apoptosis in them. Furthermore, the real-time PCR findings demonstrated that crocin enhanced the expression of the *PTEN* gene (P=0.04) in MCF7 cells, while significantly reduced the expression of the *Akt1* gene (P=0.03) (Hajizadeh and Ghorbian, 2022). miR-122-5p potential targets were identified through starbase, Targetscan, and miRDB software. A luciferase reporter assay was used to confirm the directing of sprouty2 (SPRY2) and forkhead box P2 (FOXP2) by miR-122-5p. In tissues and cells affected by breast cancer, miR-122-5p was upregulated. Crocin inhibited miR-122-5p to prevent breast cancer cells from proliferating. In breast cancer, seven miR-122-5p targets were found.

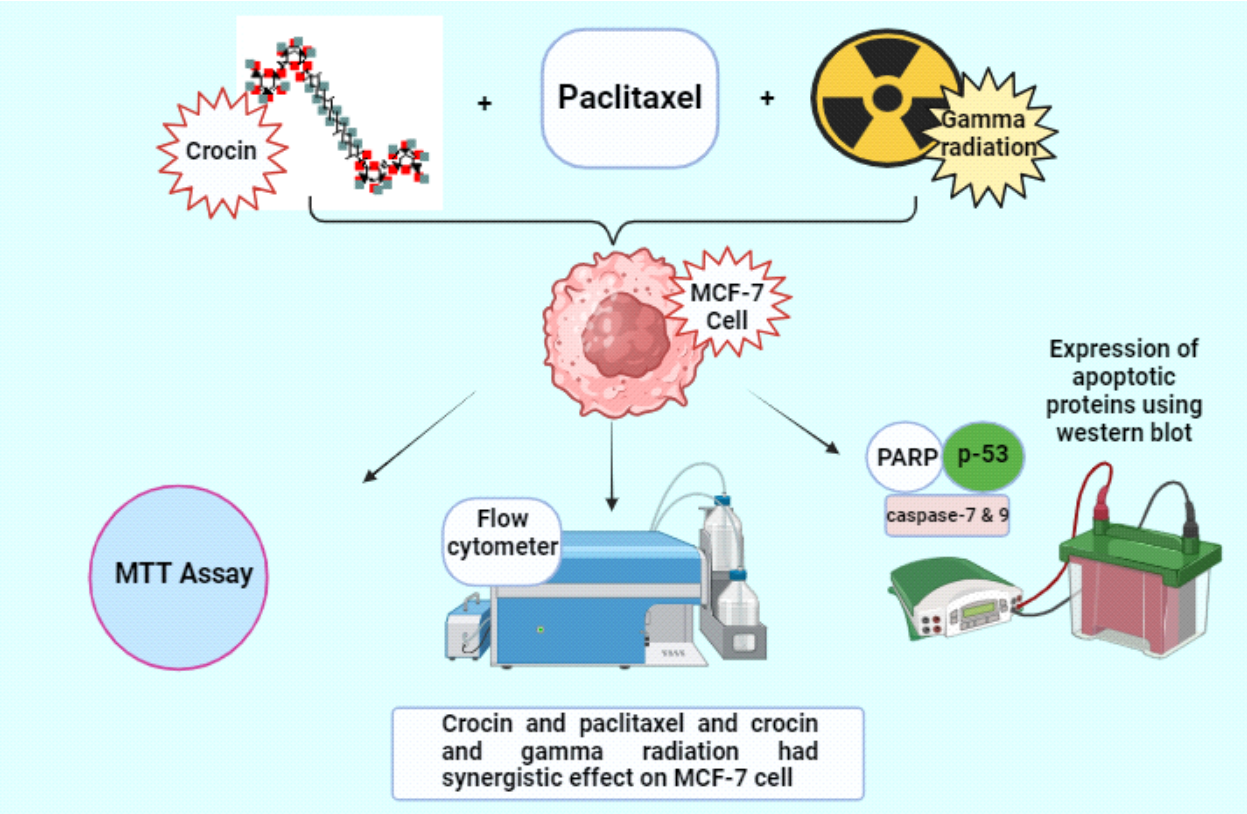

Figure 2: Synergistic Apoptotic Effect of Crocin and Paclitaxel or Crocin and Radiation on MCF-7 Cell.

Because SPRY2 and FOXP2 are implicated in breast cancer, they were chosen for additional research. Moreover, crocin was observed to enhance SPRY2 and FOXP2 expression by suppressing miR-122-5p expression. Subsequently, the findings suggest that crocin hindered the breast cancer cells proliferation by decreasing miR-122-5p expression and consequently enhancing SPRY2 and FOXP2 (suppressors of tumor) expression (Jia et al., 2023). N-nitroso-N-methylurea (NMU)-stimulated breast cancer in rats was used to study the effect of crocin on cell cycle regulators. The results showed that tumor volumes in the control and crocin-treated groups were 23.6±8.8 and 11.9±2.2, respectively, at the research end, compared to their pre-treatment values of 13.27±3.77 and 12.37±1.88. An analysis of the pathology revealed that NMU caused the induction of adenocarcinomas. Crocin treatment inhibited the expression of p21Cip1 and cyclin D1. It has been demonstrated that crocin inhibits the growth of cancers and causes cell cycle arrest via cyclin D1 downregulation. Furthermore, this herbal remedy inhibited p21Cip1 in a way that was dependent on p53 (Ashrafi et al., 2015).

4. Help to sensitizing breast cancer cells to drug

TNBC MDA-MB-231 breast cancer cells exhibit concentration-dependent apoptosis in response to crocin. The impact of doxorubicin (DOX) and crocin-loaded liposomes on the MDA-MB-231 cell line demonstrated that crocin markedly increased DOX's cytotoxicity, and crocin-loaded liposomes further increased the cytotoxicity. Furthermore, DOX plus crocin-loaded liposomes increased the number of cells in the Sub-G1 and G2/M phases. Compared to the control, treatment with crocin-loaded liposome resulted in a significant down regulation of cyclin-B1, survivin, and Bcl-xl mRNA levels and an up-regulation of Bid and Bax mRNA. Thus, crocin formulation into liposomes can be viewed as a promising strategy against tumor cells, particularly in conjunction with DOX (Chavoshi et al., 2023). Research on the effects of

crocin and metformin together on the 4T1 cell line used to study metastatic breast cancer revealed that both medications decreased cell viability, slowed the healing of scratches and inhibited cell adhesion in vitro. The mice's weight reduction was restored by crocin alone, but in a murine breast cancer model, the combination of crocin and metformin significantly decreased the size of the tumor and increased the animal survival rate. These effects were linked to the down-regulation of VEGF (angiogenesis) and MMP9 (migration) factors (Farahi et al., 2021). One of the first-line chemotherapy drugs used to treat breast cancer is called paclitaxel (PTX). Crocin made MCF-7 and MCF-7/PTX cells more susceptible to the PTX-stimulated reduction in viability and enhance in apoptosis. Crocin's target against breast cancer has been determined to be BIRC5. Crocin hindered MCF-7 and MCF-7/PTX cells from expressing BIRC5. As well as PTX-sensitive and PTX-resistant breast cancer cells, BIRC5 is overexpressed in breast cancer tissues. Breast cancer patient survival is negatively correlated with BIRC5 expression. In both MCF-7 and MCF-7/PTX cells, depletion of BIRC5 exacerbated the viability reduction caused by PTX and promoted apoptosis. Additionally, BIRC5 overexpression eliminated crocin's inhibitory effect on breast cancer cells' PTX resistance. Ultimately, crocin inhibited BIRC5 expression, which improved the sensitivity of PTX in breast cancer cell line (Jia et al., 2024).

5. Other effects

The effect of Crocin on anxiety, depression, and chemotherapy toxicity in breast cancer patients undergoing doxorubicin-based chemotherapy has been performed via the entrance of the seventy-two patients in the study with triple negative breast cancer or non-metastatic Her2/neu positive. They were divided into two groups, with one group receiving 30 mg/day of Crocin and the other group receiving a placebo during chemotherapy. The results revealed that the Crocin group experienced a significant decrease in both anxiety and depression levels ($p = .001$ for both). In contrast, the placebo group showed a significant increase in anxiety ($p = .006$) and depression ($p = .036$) rates. These findings suggest that Crocin administration during chemotherapy may have a positive influence on reducing anxiety and depression in breast cancer patients. The crocin group showed a marked increase in grade II-IV leukopenia, while the placebo group experienced grade II-IV hypersensitivity responses as well as neurological disorders. These findings suggest that the use of crocin alongside chemotherapy in breast cancer patients has helped alleviate feelings of depression and anxiety (Salek et al., 2021).

Research on the impact of crocetin (Crt) / crocin (Cro) on serum and tumor tissue triglyceride (TG) and cholesterol (Chl) levels was conducted using 4T1-induced breast cancer in mice. Additionally, following Crt/Cro remedy, the Chl/TG levels in the MCF-7 and MDA-MB-231 cell lines cytosol were measured. Results indicated that Crt and Cro successfully reduced the amount of Chl/TG in breast cancers, breast cancer cell lines, and the sera of tumor-bearing mice. The potential for hypolipidemia was greater in Crt than in Cro. The HMGCR active site appeared to bind Crt, according to in silico analysis (Hashemi et al., 2020).

Crocin's cardio protective effect has been studied in two clinical trials in breast cancer patients undergoing neoadjuvant chemotherapy (NCT06187818) and radiotherapy plus chemotherapy (NCT05504148). (Wang et al.). These studies are being completed.

6. Conclusion

Crocin induces HUVEC inhibition. Moreover, after treatment with crocin, there is a decrease in the expression of CD34 in tumor tissues, leading to anti-angiogenesis. Furthermore, the anti-angiogenic effect of crocin can hinder vascular endothelial cell proliferation, migration, and tubule formation in vitro, as well as tumor cell proliferation and reduction in microvascular density in vivo. Crocin has displayed inhibitory effects on inflammation and proliferation via NF-κB pathway in breast cancer cells by decreasing PRKCQ expression. Furthermore, it elevates the expression of the PTEN gene linked to apoptosis. The combination of paclitaxel, crocin, and gamma radiation had a strong synergistic impact on MCF-7 cells, resulting in substantial apoptosis. Ultimately, crocin increases the sensitivity of PTX in breast cancer cell lines. Crocin has been shown in preclinical research to have synergistic effects with common chemotherapy medications (e.g., doxorubicin, paclitaxel), however there is limited information on the best combinations, dose, and timing. Crocin's poor bioavailability in vivo may limit its usefulness in combination therapy, perhaps lowering pharmacological synergism. Crocin's

anti-angiogenic properties, like its antiproliferative effects, require larger concentrations in animal models, raising concerns regarding its viability in clinical application. Most crocin investigations are currently in the preclinical (in vitro and in animal models), with few clinical trials to demonstrate efficacy and safety in humans. Crocin interactions with other medications have the potential to produce unexpected results, necessitating additional investigation.

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