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## Saffron: From flavour to anti-cancer

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### Abstract

Cancer is the major cause of death worldwide and existing treatments like radiotherapy and chemotherapy have various side effects and also, are not found to be 100% successful. This has led to the need of alternate treatment options with minimum side effects. Many common Indian spices and herbs in Indian ayurvedic medicine such as ginger, turmeric, cumin, basil, saffron are known to have anticancer potential. Saffron is the dried stigmas of *Crocus sativus* L. plant. It consists of about 150 components out of which the more powerful phytochemicals are Crocin, Crocetin and Safranal. These carotenoids appear to suppress cell growth in neoplastic cells and offer direct protection against chemotherapy-induced DNA damage. Saffron causes cell cycle arrest and DNA fragmentation by down-regulation of signalling genes and up-regulation of suppressors. The objective of this review is to show that this spice with ancient origins can be used in the treatment of many different types of cancers such as lung cancer, skin cancer, liver cancer, leukemia, colorectal cancer, cervical cancer, pancreatic cancer and breast cancer.

**Keywords:** Saffron, anticancer, Crocin, Crocetin, Safranal

### 1. Introduction

Cancer is defined by uncontrollable division of cells that destroy body tissue. According to World Health Organization (WHO) that, cancer major cause of death or morbidity in human population (Globocan, 2016) [7]. The death toll because of cancer has increased from 7.4 million in 2004 to 8.2 million in 2012 worldwide as reported by the International Agency for Research on Cancer (IARC). (Globocan, 2016) [7]. Incidences of cancer are being observed to increase, recently and this is mainly attributed to urbanization, industrialization, lifestyle changes, increased population and increased elderly population. (Zanardi *et al.*, 2016) [5]

Recently great emphasis has been given on alternative medicines as the existing treatments for cancer like, radiotherapy and chemotherapy are not very promising and have various side effects. Now a day's traditional knowledge has also been utilized for the development of new effective medicines. Many common Indian spices and herbs in Indian ayurvedic medicine such as ginger, turmeric, cumin, basil, saffron are known to have anti-cancer potential. (C.M. Kaefer and J.A. Milner, 2011) [4]

Saffron is one of the most expensive spices in the world. Iran is the leading producer of saffron followed by India and Spain. In addition to its culinary use it has a history of being used in traditional medicine for centuries. Saffron (Figure 1.) is the dried stigmas of *Crocus sativus* L. plant. It belongs to family Iridaceae and its classification of the plant is listed in Table 1. It consists of about 150 components out of which, the most powerful phytochemicals are Crocin, Crocetin and Safranal. These phytochemicals are responsible for Saffron's exclusive colour, taste and odor. (John *et al.*, 2010) [15] (S. Saeed and B. Abasalt, 2014) [19] In addition to taste enhancement, saffron also demonstrates many health benefits such as: antioxidant, anti-inflammatory, anti-mutagen, anti-genotoxic, chemo-preventive and tumoricidal activity. (F.I. Abdullaev and J.J. Espinosa-Aguirre, 2004) [6]

Studies on animal models and malignant human cell lines have shown that saffron extract possess chemopreventive abilities that inhibit tumor formation and prevents chemotherapy – induced mutations that activates cancer genes and cause DNA damage. (W Gutheil *et al.*, 2012) [20] (M. Giaccio, 2004) [12] One of the most important components of saffron is Crocetin.

It inhibits nucleic acid synthesis which affects the growth of cancer cells by, inducing apoptosis and cell cycle arrest, enhancing anti-oxidative system and hindering pathways involved in growth factor signalling. (Gutheil *et al.*, 2012) [20] Saffron also help storeducethe chance of development of new cancers and harmful effects of radiation and chemotherapy. (K. Premkumar, 2006) [10] Analysis of the effect of saffron extract on various cell lines signify the need to consider saffron as a safe option for anticancer medicine.



**Fig 1:** Dried stigmas Saffron

**Table 1:** Classification of *Crocus sativus* (Saffron)

Kingdom	Plantae (Angiosperms, Monocots)
Order	Asparagales
Family	Iridaceae
Subfamily	Crocoideae
Genus	<i>Crocus</i>
Species	<i>C.sativus</i>

## 2. Lung cancer

The anticancer activity of the aqueous extract of saffron was studied against human alveolar adenocarcinoma (A549) cells. Investigation showed that saffron has the potential to induce cytotoxicity and apoptosis in cancer cells. After 48 hours of treatment of the A549 cells with different concentrations (200, 400, and 800  $\mu\text{g}/\text{mL}$ ) of saffron extract, cell viability was measured by MTT assay and morphological studies were carried out. The extract of saffron showed significantly high growth inhibitory effects on A549 cell line in a dose and time dependent manner. After 72 hours, again aqueous extract decreased cell viability. Observations under inverted microscope indicate change in morphology of the cells which were treated with higher dose (400  $\mu\text{g}/\text{mL}$ ) of saffron. They had more prominent growth inhibition and shrinkage compared with those treated with a lower dose (200  $\mu\text{g}/\text{mL}$ ). The dose inducing 50% cell growth inhibition ( $\text{IC}_{50}$ ) against A549 cells was  $380 \pm 9.6$  and  $170 \pm 2.5$   $\mu\text{g}/\text{mL}$  after 48 and 72 h, respectively. To further confirm the role of caspases in apoptotic activity of saffron extract, a potent fluorescent-labeledcaspase inhibitor that covalently binds to active caspase within the cells was used. Results showed that caspase-dependent pathways have a major role in saffron-induced apoptosis as some of the caspases were found to be activated in the presence of saffron.

## 3. Pancreatic cancer

A study explained the initiation of apoptosis by Crocin, in BxPC-3 (human pancreatic cancer) cell line. The evaluation of the viability of cells was done using MTT assay. (Hamid *et al.*, 2010) [2] Crocin led to the apoptosis of BxPC-3 cells, at 10g/L and the cell viability was reduced in a time dependent manner. The apoptosis was significantly increased after 72hrs. Whereas, within incubation time (48hrs) only decrement in cell viability was observed. Treating BxPC-3 cells with Crocin resulted in the arrest of cell cycle at G1 phase after 48 hrs which was less prominent. The increment of sub G1 fraction cells after 72 hrs was more recognizable. The apoptotic pattern was discovered within 72 hrs of incubation using Hoechst staining which shows that there might be apoptosis of BxPC-3 human pancreatic cancer cells because of Crocin-induced accumulation of cells in G1 stage. A time and dose dependant DNA fragmentation was studied in Bx-PC-3 cells treated with Crocin. The results from dose-response studies showed that for the induction of fragmentation in Bx-PC-3 cells,  $\text{IC}_{50}$  concentration of 10 $\mu\text{g}/\text{ml}$  of Crocin was optimum. The typical DNA "Ladder" pattern in Bx-PC-3 cells after treating it with 10 $\mu\text{g}/\text{ml}$  of Crocin (from 24h to 36 hours) was indicated from time point studies (0 to 36 hours). This experiment shows that Crocin has the potential to work against pancreatic cancer. (Hamid *et al.*, 2010) [2]

## 4. Colorectal cancer

One of the most frequent malignancy worldwide, "colorectal cancer" is a type of adenocarcinoma. It often begins as a polyp, which is formed on the inner wall of the colon or rectum. Experiment was conducted to predict anti-proliferative effect of pure saffron in human colon cancer HCT116 cell lines obtained from ATCC.

Cell lines were treated for 24 and 48 hours under various concentrations of saffron. Further, dose-dependent effect on cell proliferation was assessed using MTT assay. Saffron extract prepared from 100 mg/ml stock solution was used overnight to treat living cells. Proliferation of HCT116 cells was significantly reduced in a time and concentration dependent manner using saffron. Remarkable effects were seen when the concentration was between 2 and 4 mg/ml. HCT116 p5 cells tend to released cells into G2/M phase without increase in Pre-G1 apoptotic cell fraction which showed that saffron also induced p53- dependent cell cycle arrest. This arrest was reflected by HCT116 p53 wildtype cells followed by double S-phase peak in cell lines. Data from this study suggests that saffron shows a p53-dependent efficacy thus, saffron being a popular herbal spice is a viable option in the treatment of colorectal cancer. (K. Bajbouj, 2012) [11] (M. Mousavi, 2014) [13]

## 5. Breast cancer

A study was conducted with saffron on breast cancer, a leading cause of cancer in women. In a study by Mousavi saffron was freeze dried and an aqueous extract was prepared. MCF7 cells were grown in RPMI 1640 medium. The cells were treated with different concentrations (100, 200, 400 and 800  $\mu\text{g}/\text{ml}$ .) of saffron extract. After isolating total RNA and performing cDNA synthesis, real time PCR was performed to analyze the expression level of VEGFR2. Results were such that, the saffron extract showed most reduction in the expression at 100  $\mu\text{g}/\text{ml}$ , also the electromagnetic field reduced VEGFR2 up to 25%. An

inhibitory effect of saffron on angiogenesis was also indicated in the study which plays a crucial role in pathological conditions such as tumour growth and metastasis (Mousavi, 2014) [13]. It was suggested that saffron showed inhibitory effects on VEGFR2 gene expression in MCF7 cells it may be used as chemotherapeutic agent for cancer. (Mousavi, 2014) [13]

## 6. Lukemia

Many studies provided evidences that saffron exerts chemopreventive effect against Human Leukemia Cells (HL-60). Crocin, has shown inhibition in proliferation of HL-60 cells using MTT assay (Y. Sun, 2013). Dose and time dependent inhibitory effect of Crocin (0.625-10mg/mL) was observed on HL-60 cells. The initiation of apoptosis and cell cycle arrest was seen after treating the cells with Crocin. Flow cytometry using PI staining was done to examine whether Crocin inhibits the proliferation. The G0/G1 cells increased from 55.33% in control group to 70.27% in the Crocin-treated group (at 5.0 mg/mL). Whereas, at 10 mg/mL, no increment of G0/G1 cells was observed. This tells that cell cycle arrest at G0/G1 was initiated by Crocin. Acridine orange/ethidium bromide (AO/EB) staining showed that control HL-60 cells were green live cells and had normal morphology but after the treatment of cells with 0.625–2.5 mg/mL Crocin, green early apoptotic cells with nuclear margination and chromatin condensation were seen. Treating with higher concentration of (5 mg/mL) Crocin, cells went for apoptosis fragmenting chromatin. Apoptotic bodies were observed in this case. At 10 mg/mL Crocin cell adopt necrosis in place of apoptosis. This proves that Crocin can initiate the apoptosis of HL-60 cells.

*In vivo* experiments were also performed to check the anti-cancerous activity of Crocin (P.A. Tarantilis, 1994) [14]. The introduction of HL-60 cells into mice led to the decrease food ingestion and spontaneous activity were also reduced for all mice. The difference in the body weight of the four groups was not seen at the time they received HL-60 cells. The rate and time of formation of tumor of the control and experiment groups at 6.25, 25, 100 mg/kg Crocin were 100%, 50%, 75%, and 75% and 11.50 ± 1.60, 20.00 ± 1.15, 14.30 ± 1.86, and 10.50 ± 1.64 d respectively. There was no difference in the rate of formation of tumor among the four groups. The results showed that the formation of xenograft (with HL-60 cell) could be slowed down in nude mice at 6.25 and 25 mg/kg Crocin. This concludes that Crocin can inhibit the growth of HL-60 cell xenograft in nude mice.

In another study the immunohistochemistry analysis of Bcl-2 and Bax expression in xenograft was done to check whether the suppression of growth of tumor by Crocin is due to the initiation of (B.C. Cavalcanti, 2009) [3]. There was an increase in the number of Bax positive cells and decrease in the number of Bcl-2 positive cells in tumors from mice treated by 6.25 or 25 mg/kg Crocin, compared to those from controls. Western blot analysis was also done of Bcl-2 and Bax expression in xenografts. The decrement in the protein level of Bcl-2 and increment in the protein level of Bax was seen in tumors derived from mice treated with 6.25 or 25 mg/kg Crocin, compared to those from control. Thus, it designates that Crocin can reduce Bcl-2 expression and increase Bax expression which leads to the increased apoptosis in HL-60 cell xenograft. (Y. Sun, 2014) (P.A. Tarantilis, 1994) [14] (B.C. Cavalcanti, 2009) [3]

## 7. Liver cancer

Scientists are trying nanotechnology also along with these traditionally known herbs. In a study, the researchers coated magnetite nanoparticles with saffron components like Crocin and injected them into mice and human liver cell line i.e. HepG2 cells (R.E. Kharrag, 2016) [16]. The conjugation of Crocin was enhanced using dextran and cross linkers. Examination of the result was done with cell proliferation assay and then immunohistochemical analysis was performed. Inhibition of cancerous growth of HepG2 cells was observed in the cell line where saffron coated magnetite nanoparticles were used while, non-coated nanoparticles showed no response. Histological examinations of the livers of injected mice revealed several pre-cancerous changes including multiple proliferative mutated hepatic cells, hyper- or dysplastic transformations of bile ducts/ductules, and nuclear atypia associated with polyploidy, nucleus enlargement, and vacuolation. Immunohistochemistry was performed using specific antibodies for inflammation (cyclooxygenase-2), oxidative stress (glutathione) and angiogenesis (vascular endothelial growth factor) where development of pre-cancerous lesions involved multiple signaling pathways. All in all, saffron was successful in inhibiting the cancer growth in liver cell line. (R.E. Kharrag, 2016) [16]

## 8. Cervical cancer

A study was conducted using HeLa cell line (human cervical cancer) to check the cytotoxic activity of saffron. HeLa cell lines were incubated at different concentrations of saffron for 24hrs and at different concentrations of crocin (0.5 and 1 mM). It was Followed by MTT assay which showed decreased cell viability with increasing crocin concentration. Another study conducted by Mousavi *et al.* In 2011 [18] reported similar results proving that saffron and its components helped in cytotoxicity of cells (Mousavi *et al.*, 2011) [18]. Also, have published that Saffron has cytotoxic effect on HeLa cell line which included programmed cell death. The cell viability was decreased in malignant cells as a concentration of saffron (IC50 value 800 and 950 microgram/ml) and time-dependent (48hrs) manner. Another *in vitro* study reported that cells treated with Crocin, resulted in wide cytoplasmic vacuole-like areas, cell shrinkage, reduced cytoplasm, and pyknotic nuclei, indicating that apoptosis took place. (J. Escribano, 1995) [9]

## 9. Skin cancer

In a study carcinogenesis was induced into the carcinogen control group (CC groups) mice. They were given three topical applications of 100 n molDMBA (7-12 dimethylbenz [a] anthracene) in 100µl acetone at an interval of 2 days on the shaved skin. After a week at the same site 100µl 1 gm% croton oil in acetone was given twice weekly for 8 weeks. Another group was normal control groups (NC groups) which received distilled water every day during the treatment period. (I. Das, 2004) [8] Upon injection of saffron to both the groups, the body weights of all the mice gradually increased upto 8 weeks excluding those from the carcinogen control groups (CC groups). There was significant difference in the body weight between the normal control groups (NC groups) and carcinogen control groups (CC groups) after 12 weeks. Normal values were seen in the treatment groups A and B. Saffron also affected development of papillomas. Pappilomas were seen from the

5th to 6th weeks on the region of skin receiving topical application of DMBA (7-12 dimethylbenz [a] anthracene) and croton oil. The papilloma continued to grow in mice till 12th week in the CC group. The growth of papilloma in the CC group after the treatment for 12 weeks was 90%. There was reduction in the growth of papilloma in all the groups. The growth of papillomas slowed down from the 5th week to the 7th week in Group A and Group C and to 9th week in Group B (Set II animals). Saffron thus affected the growth of papillomas. The most significant effect of the saffron was observed in Group B.

Saffron prevented lipid peroxidation (LPO) and reduced glutathione S transferase activity (GST activity), glutathione peroxidase activity (Gpx activity), catalase activity (CAT activity) and superoxide dismutase activity (SOD activity) in CC than NC. (I. Das, 2004)<sup>[8]</sup>

## 10. Conclusion

Worldwide researchers are working on the correlation of saffron with lowering risk of many types of cancers like lung cancer, skin cancer, liver cancer, leukemia, colorectal cancer, cervical cancer, pancreatic cancer and breast cancer. They are also investigating the contribution of the large number of phytochemicals in saffron. Among these phytochemicals, Crocins, Crocetin, and Safranal are considered the most pharmacologically advantageous as they affect cell growth regulation, and modulate gene expression in cancer cells. Several *in vivo* and *in vitro* studies have examined the use of saffron extract in cancer prevention and therapy. Recent findings have not yet been able to explain the exact mechanism of anticancer effects of saffron. This review suggests that saffron's anti-cancer activity needs to be further studied to provide impediments to human chemoprevention and cancer treatment.

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