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The Potential Role of Saffron Extract, Crocin, and Crocetin in Modulating TNF-*α* And IL-6 Levels in Various Diseases: A Review

Lani Ishak[1], Diana K. Jasaputra1[1,2], Julia W. Gunadi[1,3*], Lukas M. Samuel[4], Rizna T. Rumanti[5]

[1]*Master Program of Skin Ageing and Aesthetic Medicine, Faculty of Medicine, Universitas Kristen Maranatha, Bandung, West Java 40164, Indonesia*
[2]*Department of Pharmacology, Faculty of Medicine, Universitas Kristen Maranatha, Surya Sumantri 65, Bandung, West Java 40164, Indonesia*
[3]*Department of Physiology, Faculty of Medicine, Universitas Kristen Maranatha, Surya Sumantri 65, Bandung, West Java 40164, Indonesia*
[4]*Department of Internal Medicine, Faculty of Medicine, Universitas Kristen Maranatha, Surya Sumantri 65, Bandung, West Java 40164, Indonesia*
[5]*Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Kristen Maranatha, Bandung, Surya Sumantri 65, Bandung, West Java 40164, Indonesia*

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ABSTRACT

Increasing interest has been directed toward saffron (*Crocus sativus*) and its active ingredients, crocin and crocetin, due to their potential to reduce inflammation by modulating cytokines such as tumor necrosis factor alpha (TNF-*α*) and interleukin-6 (IL-6). Unlike synthetic drugs, which can be associated with adverse side effects, phytochemicals such as saffron present a potentially safer alternative for managing inflammation-related conditions. This review aims to evaluate the effects of saffron, crocin, and crocetin on lowering TNF-*α* and IL-6 levels. A literature review was conducted on experimental studies published during the past 10 years. Articles were sourced from PubMed and Google Scholar using the keywords "crocus," "crocin," "crocetin," "TNF-*α*," and "IL-6," adhering to the inclusion criteria. Sixteen studies meeting these criteria were examined. Both *in vivo* and *in vitro* studies indicate that saffron extract and its components reliably decrease TNF-*α* and IL-6 levels in various inflammatory situations. Nevertheless, results from randomized controlled trials are inconsistent, with some research demonstrating significant reductions in these cytokines, while others reveal negligible or no effects. Such discrepancies suggest that the advantages of saffron and its components may depend on variables such as dosage, research methodology, and patient characteristics. Saffron and its derivatives exhibit potential as natural anti-inflammatory agents; however, further extensive, high-quality randomized controlled trials are necessary to confirm their therapeutic efficacy, safety, and mechanisms of action. The promise of saffron as a treatment for mitigating pro-inflammatory cytokines, including TNF-α and IL-6, is evident, yet stronger scientific evidence is still required.

Keywords: Crocetin, Crocin, Interleukin-6, Saffron, Tumor Necrosis Factor-Alpha

Introduction

Tumor necrosis factor-alpha (TNF-*α*) is a significant cytokine involved in the immediate inflammatory reaction in numerous cell types. TNF-*α* production is correlated with physiologic immune response, but overproduction may lead to harmful effects.[1,2] Physiologically, TNF-*α* is important in pathogen defense, the formation of germinal center (i.e., the spleen), including resolution and repair of inflamed tissues.[3] Nevertheless, TNF-*α* overproduction has been linked to inflammation, immune cell recruitment, and tissue disintegration that eventually lead to the development of diseases.[1,3] Similarly, Interleukin-6 (IL-6) is a soluble mediator that shows diverse effects on inflammation, immunological response, and hematopoiesis.[4] IL-6 is physiologically induced during the acute response to infections, aiding in the immune defense against pathogens; however, its dysregulated production can contribute to chronic conditions such as rheumatoid arthritis, diabetes, colitis, cancer, and others.[4–7]

*****Corresponding author. E mail: julia.windi@maranatha.ac.id

Tel: 022-2012186

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Although synthetic medications are commonly used to treat established diseases, traditional medicine and the utilization of phytochemicals such as propolis and saffron have garnered significant interest from the medical community.[8,9] The reason for this is that traditional remedies are more cost-effective, readily accessible, and exhibit fewer adverse effects compared to contemporary pharmaceuticals.[10] Belonging to the Iridaceae family, saffron is a medicinal plant scientifically identified as *Crocus sativus L.* It grows in many regions of Asia, Africa, and Europe.[11] The plant is perennial, with a height of 30 cm, long and smooth leaves, and purple-cup-shaped flowers. It is resistant to sunshine and temperatures below 15°C, and thrives on clay and calcareous soil with a pH ranging from 6 to 7.[12] The saffron pistil consists of three reddish-orange stigmas that emit a pleasant odor.[13] Once the blooms are manually harvested, the stigma is isolated, dehydrated, and utilized as saffron.[14]

Volatile and nonvolatile chemicals in saffron's stigma have been detected through phytochemical investigation. Saffron contains numerous essential nutrients (minerals, proteins, carbohydrates, and vitamins such as B1 and B2) and four primary bioactive compounds: crocin, crocetin, picrocrocin, and safranal.[15] Crocin and crocetin are carotenoid compounds that give saffron its yellow coloration, picrocrocin imparts its flavor, and safranal contributes to its distinctive fragrance.[12] Saffron, in addition to its role in cuisine, has demonstrated potential in mitigating numerous conditions due to its antioxidant, anti-inflammatory, anticancer, antidiabetic, and antihypertensive effects observed in animal research.[16] The primary attributes of saffron that contribute to its antioxidant and anti-inflammatory properties are mostly associated with crocin, a compound in saffron stigmas.[17]

Safranal, a monoterpene aldehyde derived from saffron essential oil, has several biological actions such as antihyperglycemic, anti-inflammatory, antioxidant, anticonvulsant, and anxiolytic effects.[13]
Saffron and its ingredients generate anti-inflammatory and immunoregulatory effects by regulating pro-inflammatory cytokines and immunological factors.[18] Physical, chemical, and biological variables of saffron may activate the inflammatory process and the immune system, which results in interactions between the two.[19] Inflammation has been demonstrated to have a crucial function in the development of different diseases and conditions, including allergies, asthma, and cardiovascular ailments.[19] Despite the extensive research on saffron's therapeutic potential, this study uniquely combines data from in vitro, in vivo, and randomized controlled trials (RCTs) to deliver a comprehensive analysis of its anti-inflammatory and immunomodulatory actions. Highlighting saffron's potential to modulate TNF-α and IL-6 in multiple illnesses, this study also addresses shortcomings in current data, providing direction for future research efforts. Despite variations in results across different studies, the aim is to provide a comprehensive analysis of the topic, with this literature review specifically examining the ability of saffron and its components to reduce pro-inflammatory cytokines (TNF-*α*, IL-6, NF-*κB*) in different body systems.

Materials and Methods

We employed a literature review to assess the anti-inflammatory properties of saffron and its components. We obtained articles from PubMed and Google Scholar using the following keywords: "crocus," "crocin," "crocetin," "TNF-*α*," and "IL-6." The review employed a systematic methodology, utilizing inclusion and exclusion criteria to guarantee the selection of pertinent and high-quality studies.
The inclusion criteria were the following: (1) Researchers conducted experimental investigations *in vitro*, *in vivo*, or clinical settings; (2) released in English; (3) research in health or related fields has been published in the past decade; and (4) access to full text. The exclusion criteria included the following: (1) The study lacks comprehensive methodological details or sufficient data for assessment; and (2) consensus papers, literature reviews, and redundant studies. Sixteen research studies fulfilled the inclusion criteria and were meticulously examined to evaluate the efficacy of saffron and its components in reducing pro-inflammatory cytokines, specifically TNF-*α* and IL-6. This methodical approach guarantees thorough coverage and dependable results, in accordance with established literature review norms.

Results and discussion

Saffron, especially its stigma, has over 150 different compounds.[20] Its strong antioxidant properties have been proven by its ability to decrease pro-inflammatory enzymes (cyclooxygenase 2, phospholipase A2, etc.), as well as its capability as an NF-*κB* antagonist and a peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist.[18] The modulation of NF-*κB* and PPAR-γ signaling by saffron and its components plays a crucial role in regulating pro-inflammatory cytokines, particularly TNF-α and IL-6.[18,21] High concentrations of saffron compounds (crocin, crocetin, and safranal), which possess potent antioxidant properties, could effectively neutralize free radicals, thus reducing the inflammatory process in various diseases.[21–24] Several scientific studies have been carried out to investigate the characteristics of this plant in its ability to reduce pro-inflammatory cytokine levels, focusing on several components of *Crocus sativus*, such as crocin, safranal, and crocetin.[18,25,26] What follows is an overview of 16 studies that demonstrate the role of saffron extracts, crocetin, and crocin in lowering pro-inflammatory cytokine levels, especially TNF-*α* and IL-6.
The potential role of saffron extract in modulating TNF-*α* and IL-6 levels in various diseases
This study found eight original articles regarding the potential role of saffron extract in modulating TNF-*α* and IL-6 levels in various diseases (see Table 1).

Research conducted by Rathore *et al*.[27] involving 36 female Swiss albino mice, clinically demonstrated that the group of animals treated with *Crocus sativus* extract showed a significant decrease in TNF-*α*. From Day 1 to Day 47, *Crocus sativus* extract was administered to the animal groups at doses of 25, 50, and 100 mg/kgBB. On Day 47, there was a decrease in inflammation in the group of animals given the extract. Active compounds such as crocin, crocetin, safranal, and flavonoids are believed to be responsible for the anti-inflammatory and antioxidant effects of *Crocus sativus* extract. The presence of the extract is believed to be responsible for the existence of all active ingredients. The various active components are believed to inhibit free radicals and offer protective benefits against oxidative harm. The administration of *Crocus sativus* extract effectively decreased the levels of pro-inflammatory cytokines, including TNF-*α* and IL-1β, in a group of rats with induced rheumatoid arthritis. The effectiveness of this extract suggests the presence of anti-inflammatory effects.[28]
According to a study conducted by Shahbazian *et al*.,[21] a notable rise in TNF-*α* and IL-6 levels was observed in both the saffron and placebo groups during the 3-month study period. Sixty-four patients with type 2 diabetes mellitus (T2DM) were included in this study. The patients were segregated into two distinct groups (1:1 ratio), namely, those receiving saffron (intervention group) and placebo (control group). In the intervention group, patients were administered two 15 mg capsules of saffron, whereas in the control group, they were given two placebo capsules. This treatment regimen lasted for 12 weeks.
A hallmark of T2DM is a concealed systemic inflammatory response, largely due to increased levels of IL-6 and TNF-α in the blood plasma. By disrupting insulin secretion and reducing the activation of insulin receptors, TNF-α significantly contributes to the onset of insulin resistance in humans. Nevertheless, the findings of this study indicated that saffron did not yield any advantageous outcomes in terms of oxidative stress and inflammatory markers among individuals with T2DM.[29] Unlike other studies, these results highlight discrepancies, prompting the authors to recommend additional investigations involving larger sample sizes and increased saffron doses to validate its effectiveness and mechanism in T2DM management.
These findings are inconsistent with prior studies, which propose that saffron's active components significantly reduce inflammation and suppress the production of inflammatory mediators linked to insulin resistance. A study by Mobasseri *et al*.[26] revealed a substantial drop in serum IL-6 and TNF-*α* concentrations following saffron ingestion. This study was a RCT that included 60 individuals diagnosed with T2DM. Thirty participants in Group A were given saffron, while another 30 in Group B received a placebo. Both groups took one daily capsule containing 100 mg of saffron powder or starch for an 8-week duration. Saffron is believed to have the ability to decrease the inflammatory effects associated with diabetes by reducing the levels of TNF-*α*, IL-6, and IL-1β. The outcomes of this study suggest that this therapy could emerge as a novel and effective option for treating inflammatory conditions, particularly diabetes.[26]
The research conducted by Samarghandian *et al*.[22] demonstrated that administering saffron had a significant dose-dependent effect on decreasing the synthesis of TNF-*α* and IL-6 mRNA in rats with diabetes. The study comprised 45 adult male Wistar albino rats, with saffron extract administered via injection starting three days after streptozotocin (STZ) treatment and continuing throughout the 4-week trial. The precise anti-inflammatory mechanism of saffron remains uncertain, but it is theorized to regulate glucose-induced pro-inflammatory mediator synthesis. The results align with prior studies that suggest saffron hinders the activation of inflammatory substances by obstructing TNF-*α* and IL-6 in the endothelium of the abdominal aorta.[30]
Prior research points to the anti-inflammatory potential of saffron and its active ingredients, particularly their role in lowering levels of pro-inflammatory cytokines. It has been suggested that saffron could potentially serve as an effective treatment for arthritis by acting on the cyclooxygenase pathway. This effect is achieved through the inhibition of cyclooxygenase 1 and cyclooxygenase 2 enzymes, and the dosage-dependent decrease of prostaglandin E (2) synthesis was also observed. However, the findings of this study contrast those of Hamidi *et al.* ,[23] who demonstrated that the utilization of saffron resulted in a drop in

TNF-*α* levels, albeit not in a statistically significant manner. Sixty-six female patients with rheumatoid arthritis participated in this study, divided into an intervention group receiving 100 mg of saffron daily and a placebo group given hydroxypropyl methylcellulose tablets at the same dose. Following 12 weeks of treatment, reductions in TNF-α, IFN-γ, and malondialdehyde levels were observed, alongside improved antioxidant capacity, although the differences between groups were not statistically significant. To improve the accuracy of future human studies, markers such as nuclear factor kappa light chain enhancer of activated B cells and its receptor in synovial tissue, as well as serum peroxisome proliferator-activated receptor gamma, should be analyzed.[26]

According to Christodoulou *et al*.,[24] the use of saffron was found to be linked to a notable reduction in cytokine pro-inflammatory levels. The effect was more noticeable in the group that received a higher dosage. This study comprised a cohort of 25 male rats. The dried aqueous extract of *Crocus sativus L.* was given to rats at 60 mg/kg body weight via gavage. Increased doses led to a more uniform aortic endothelium plaque phenotype compared to the control group and correlated with reductions exceeding 30% in IL-6 and TNF-α levels..[24]

Roustazade *et al*.[25] reported a substantial decrease in the mRNA level of TNF-*α* in the hippocampus of the Stress+Safron 30 group compared to the Control, Safron 30, and Stress groups. At low doses, saffron alters the expression of the TNF-*α* gene in the hippocampus. Low saffron doses led to a significant drop in the expression of the TNF-*α* gene in the hippocampus, whereas high doses resulted in a large rise in the expression of the BDNF gene in the same brain region in the subchronic stress group. Consequently, saffron's impact lowered anxiety in the stressed group. Saffron extract may provide anti-inflammatory advantages by increasing the expression of neurotrophic factors, but only when delivered in modest quantities. The administration of crocin led to a reduction in the levels of the inflammatory mediator TNF-*α* in the hippocampus. This decrease in TNF-*α* resulted in oxidative stress. Ultimately, the treatment with crocin prevented apoptotic cell death in pyramidal cells.[25]

Xiao *et al*. conducted a study demonstrating that saffron effectively decreased TNF-*α* levels in the brains of aging mice.[28] Furthermore, the saffron administration considerably decreased the observed increase in TNF-*α* in control mice aged 8 and 16 months. The research encompassed a total of 40 female mice. For 8 weeks, the mice were given saffron aqueous extract at a dose of 80 mg/kg per day via oral administration. The control group of mice received an identical dosage of double-distilled H_2O through oral gavage for 8 weeks. Elevated levels of extracellular nicotinamide phosphoribosyltransferase (NAMPT) and alterations in cellular distribution can stimulate the release of inflammatory cytokines, such as TNF-*α*, IL-1β, IL-4, and oxidative stress factors, from microglia. This, in turn, results in an extra increase of NAMPT secretion. According to reports, saffron changes the way NAMPT is distributed in the cells of aging mice. The findings demonstrated reduced NAMPT expression in microglia and astrocytes, while NAMPT expression in neurons increased. Saffron also diminished pro-inflammatory cytokines and oxidative stress markers in elderly mice.[28]

The potential role of crocetin in modulating TNF-*α* and IL-6 levels in various diseases

We found three original articles regarding the potential role of crocetin in modulating TNF-*α* and IL-6 levels in various diseases (see Table 2). The research, conducted with CFA-induced arthritis models and RAW 264.7 macrophage cells in vitro and in vivo, revealed that crocetin significantly decreased TNF-α and IL-6 levels and downregulated NF-*κB* expression in synovial tissue, indicating its promise as an anti-inflammatory substance via cytokine and transcription factor modulation.[29] The findings of this study associate the reduction in pro-inflammatory cytokines with saffron, specifically crocetin, which has the capability to suppress NF-*κB* activation. NF-*κB* is critical in controlling the production of genes such as COX-2, iNOS, and TNF-α. During rheumatic illness conditions, IL-1*β* facilitates an elevation in white blood cell counts and triggers the production of colony-stimulating factor, granulocytes, and inflammatory macrophages. Crocetin exhibited a significant reduction in both granulocyte numbers and inflammatory macrophage migration, with the effects depending on the dosage.[29]

Song *et al*.'s study demonstrated that inhibiting NF-*κB* effectively reduces the levels of pro-inflammatory cytokines, such as TNF-α.[30] The study used human umbilical vein endothelial cell (HUVEC) samples in an *in vitro* setting. The results indicated that crocetin effectively blocked the translocation of the NF-*κB* p65 subunit to the nucleus, a critical step in NF-*κB* activation. By preventing the degradation of I*κB*-α, crocetin maintained NF-*κB* in its inactive state within the cytoplasm. Using an umbilical vein endothelial cell model, the study examined the effects of lipopolysaccharide (LPS) stimulation on *IκBα* kinase (IKK) activation via tumor TNFR and IL-1R pathways. This activation leads to the phosphorylation and degradation of the cytoplasmic *IκBα* subunit, releasing the p65 subunit to enter the nucleus and initiate the translation of pro-inflammatory cytokines. Crocetin stabilized the *IκBα*-p65-p50 complex, thus disrupting the signaling cascade.[30]

Otsu and colleagues[31] conducted an *in vitro* study using human corneal epithelial cell-transformed (HCE-T) samples. The study found that phosphorylation of NF-*κB* increased 24 hours after UV-A irradiation. However, the increase in phosphorylation was partially inhibited by a concentration of 10 mM crocetin.[31]

The potential role of crocin in modulating TNF-*α* and IL-6 levels in various diseases

We found five original articles regarding the potential role of crocetin in modulating TNF-*α* and IL-6 levels in various diseases (see Table 3).

Twenty-five mice with collagen induced arthritis (CIA) were the subjects of an *in vivo* experimental investigation organized by Liu *et al*.[32] The study revealed that the administration of crocin at various doses to the mice with CIA resulted in a reduction of pro-inflammatory cytokine levels in their serum to different extents. Rats treated with 40 mg/kg crocin in the CIA group showed the most substantial reduction in pro-inflammatory cytokines in their serum, compared to other dosages. Synovial tissue hyperplasia and immune cell infiltration were prominent in untreated CIA rats, with similar patterns observed in those given 10 mg/kg crocin. However, a dose of 20 mg/kg decreased cytokine presence in the joint cavity and controlled tissue overgrowth. The highest dose, 40 mg/kg, resulted in an absence of cytokines and a notable reduction in hyperplasia. These findings propose that crocin effectively suppresses inflammatory lesions in CIA, positioning it as a potential anti-arthritis therapy. Liu *et al*.[32] it is demonstrated that crocin, a compound found in saffron, has the ability to decrease the levels of TNF-*α* by suppressing the mRNA expression of pro-inflammatory cytokines.[32]

A study conducted by Fagot *et al*. utilized human keratinocyte cell samples (NHEKs) and human dermal fibroblasts in an *in vitro* experimental setting.[33] The study found that the administration of crocin at concentrations of 0.3 mM and 1 mM led to a substantial reduction in the generation of IL-6 and TNF-*α*. Additionally, the modulation of NF-*κB* signaling was also observed to decrease. Crocin exhibits antioxidant properties against reactive oxygen species, safeguards squalene against peroxidation generated by UVA radiation, and inhibits the production of inflammatory mediators. Crocin influenced the expression of NF-*κB*- and glycosylation-related genes.[32] A RCT was conducted by Aslani *et al*.,[34] which included 57 male patients diagnosed with chronic obstructive pulmonary disease (COPD). The study found that while the crocin administration group showed insignificant changes, the placebo group exhibited a significant increase in serum IL-6 levels following the intervention. The post-intervention levels of TNF-*α* did not show any meaningful difference between the placebo and crocin groups. The pathogenesis of COPD, a progressive disease defined by persistent airway limitation, is influenced by various variables, including inflammation, oxidative stress, extracellular matrix degradation, and apoptosis. Herbal supplements that have anti-inflammatory and antioxidant characteristics can aid in the treatment of specific chronic illnesses. The results indicate that the addition of crocin enhances the ability to exercise and improves lung functional tests in individuals with COPD by decreasing the levels of inflammatory factors in the blood. This study is limited by the fact that it only included male participants and had a relatively large sample size. Therefore, future research should be undertaken with a bigger sample size and include both male and female patients with COPD.[34]

Table 1: Research of the potential role of saffron extracts in modulating TNF-α and IL-6 levels in various diseases

No.	Reference	Research Design	Research Subject	Intervention	Variable	Results and Conclusions
1.	Rathore, B. *et al.* (2015)[27]	*In vivo*	A group of 36 female Swiss albino mice, aged 12–14 weeks and weighing 28–30 g.	For arthritis, mice were induced with Freund's complete adjuvant. Separated into six groups: Group 1: normal mice; Group 2: arthritic mice + distilled water; Group 3: arthritic mice + *Crocus sativus* extract (ECS) 25 mg / kgbb; Group 4: arthritic mice + ECS 50 mg / kgbb; Group 5: arthritic mice + ECS 100 mg / kgbb. ECS was administered orally daily until day 47. Group 6: arthritic mice + acetyl salicylic acid (ASA) 200 mg/kg bw.	TNF-*α* (ELISA from joint homogenates)	The group of animals treated with ECS showed a significant decrease in TNF-*α*. These results suggest that ECS has strong anti-inflammatory potential through suppression of TNF-*α* in joint inflammation.
2.	Shahbazian, H. *et al.* (2019)[21]	Randomized controlled trial	64 patients diagnosed with type 2 diabetes mellitus (T2DM)	The patients were separated into two groups. One group was administered saffron while the other group was given a placebo. Patients in the intervention group were administered two capsules containing 15 mg of saffron, whereas patients in the control group were given two placebo capsules. This treatment regimen lasted for 12 weeks.	IL-6, TNF-*α* (Quantified utilizing the e-bioscience kit manufactured in Germany)	Following a 3-month period, the levels of TNF-*α* and IL-6 exhibited a significant rise in both the saffron and placebo cohorts. The study findings indicate that saffron did not have any effects on inflammatory indicators in patients diagnosed with T2DM.
3.	Samarghandian, S. *et al.* (2017)[22]	In vivo	A total of 45 adult male albino Wistar rats weighing between 250 and 300 g were used.	Rats induced with streptozotocin (STZ) A group of Wistar rats was divided into five groups using random assignment: control; diabetic rats; diabetic rats + saffron extract 10 mg/kg bw/day; diabetic rats + saffron extract 20 mg/kgbb/day; diabetic rats + saffron extract 40 mg / kg / day. Saffron extract was injected starting from the 3rd day after STZ administration and continued until the completion of the 4-week trial period.	TNF-*α*, IL-6 (RT-PCR from abdominal aorta)	Administering saffron led to a significant drop in the expression of TNF-*α* and IL-6 mRNA in diabetic rats. The magnitude of this reduction varied depending on the dosage.
4.	Hamidi, Z. *et al.* (2020)[23]	Randomized controlled trial.	66 Female RA patients	The participants were separated into two distinct groups (n = 33). Intervention group received 100 mg/day saffron tablets. The placebo group received 100 mg hydroxypropyl methylcellulose placebo tablets. The study was conducted for 12 weeks.	TNF-*α* (ELISA)	TNF-α decreased but was not significant between the two groups.
5.	Christodoulou, E. *et al.* (2018)[24]	In Vivo	25 8-week-old C57BL/6J wild-type male mice	The experimental animals were categorized into groups, each corresponding to a particular time interval for sampling, namely 15, 30, 60, 120, and 240 min after treatment. The rats were administered the aqueous extract of WFI-dried *Crocus sativus* L. SFE orally using the gavage technique at a dosage of 60 mg/kg.	IL-6, TNF-*α* (immunohistochemistry from aorta)	The administration of saffron was linked to substantial decreases in IL-6 and TNF-*α* levels. The effect was more noticeable in the group that received a higher dosage.
6.	Roustazade, R. *et al.* (2022)[25]	*In vivo*	42 Adult male Wistar rats 200–250 g	The rats were assigned to six groups by a random selection procedure: Control + saline; Stress + saline; Saffron 30 mg / kgbb; Saffron 60 mg / kgbb; Stress + saffron 30 mg / kgbb; Stress + saffron 60 mg / kgbb. Saffron extract was injected into the peritoneal cavity for 7 days.	TNF-*α* (RT-PCR of hippocampal tissue)	The Stress+Safron 30 group exhibited a substantial decrease in TNF-*α* mRNA levels in the hippocampus compared to the Control, Safr 30, and Stress groups. Low dose of saffron affects hippocampal TNF-*α* gene expression.
7.	Mobasseri, M. *et al.* (2020)[26]	Randomized controlled trial	60 patients with T2DM	The patients were categorized into two groups: 30 individuals receiving saffron and 30 individuals receiving a placebo. Subjects in the saffron and placebo cohorts were given either 100 mg/day of saffron powder or starch capsules (1 capsule) for 8 weeks.	IL-6, TNF-*α* (ELISA and RT-PCR)	The amounts of serum IL-6 and TNF-*α* were dramatically decreased.
8.	Ling Xiao *et al.* (2024)[28]	*In vivo*	40 female C57BL/6J mice 3–8 months 20–25 g; 16 months, 25–30 g)	C57BL/6J mice were allocated into two groups: a control group consisting of mice aged 3, 8, and 16 months, and a saffron group consisting of animals aged 8 and 16 months. Each group contained eight mice. The trial lasted for 63 days. The mice were administered saffron aqua extract orally at a dosage of 80 mg/kg once daily for 8 weeks. The control group of mice received an equivalent amount of double-distilled H2O through oral gavage for 8 weeks.	TNF-*α* (ELISA from brain)	Saffron reduces TNF-α in the brains of aging mice. The increase in TNF-*α* observed in 8- and 16-month-old control mice was significantly reduced by saffron administration. Saffron has potential as an anti-inflammatory agent that can reduce the excessive inflammatory response in aging.

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Table 2.: Research of the potential role of crocetin in modulating TNF-*α* and IL-6 levels in various diseases

No.	Reference	Research Design	Research Subject	Intervention	Variable	Results and Conclusions
1.	Li, Y. *et al*. (2018)[29]	*In vitro*	RAW 264.7 macrophage cells	The cells were diluted and incubated and then exposed to different concentrations (12.5, 25, 50, 100, 200, and 400 µg/mL, dissolved in medium) of crocetin.	TNF-*α,* and IL-6, p-NF-kB p65, NF-kB p65 (RT-PCR from foot tissue)	Crocetin decreased the levels of TNF-*α* and IL-6, and reduced the expression of NF-*κB* in synovial tissue.
		In vivo	60 Swiss Wistar albino rats (100–125 g)	The rats were divided into six groups: Normal control + saline; Normal control + crocetin (20 mg/kg); Control arthritis + saline; Arthritis + crocetin (5 mg/kg); Arthritis + crocetin (10 mg/kg); Arthritis + crocetin (20 mg/kg); Arthritis + indomethacin (10mg/kg).		This finding indicates that crocetin has the ability to act as an anti-inflammatory substance, potentially by controlling pro-inflammatory cytokines and transcription factors.
2.	Song, L. *et al*. (2016)[30]	*In vitro*	Human umbilical vein endothelial cell (HUVEC)	HUVECs were cultured in RPMI 1640 medium (GIBCO) with Endothelial Cell Growth Kit (Lonza), and U937 cells were maintained in DMEM (GIBCO) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and streptomycin. Lipopolysaccharide (LPS) at 10 ng/mL (Sigma–Aldrich) was applied for 24 hours, with crocetin added at 1, 5, and 10 ng/mL, either combined with LPS or alone.	NF-*κB* p65 (Western Blot)	Crocetin has the ability to prevent the movement of the NF-*κB* p65 subunit into the cell nucleus, which is an important step in activating NF-*κB*. Crocetin has also been found to prevent the breakdown of I*κB*-*α*, which keeps NF-*κB* in its inactive state in the cytoplasm.
3.	Otsu, W. *et al*. (2022)[31]	*In vitro*	Cells of the human corneal epithelial cell-transformed (HCE-T)	HCE-T cells were subjected to UV-A radiation with a wavelength of 365 nm. HCE-T cells were cultured in a medium containing 1% FBS, with the option of adding 10 mM crocetin, and incubated for 1 hour.	NF-*κB* (immuno-blotting)	The phosphorylation of NF-kB was enhanced 24 hours following exposure to UV-A radiation. However, the extent of this phosphorylation was partially suppressed by the presence of 10 mM crocetin.
2.	Fagot, D. *et al*. (2018)[33]	Experimental *in vitro*	NHEKs human keratinocyte cells and human dermal fibroblasts	The preventative and healing properties of crocin were evaluated at doses of 0.3 mM and 1 mM. To achieve preventive effects, crocin was introduced into the cell media 2 hours prior to the formation of the inflammatory state and continued to be administered during the induction process. Crocin was administered during the initiation of inflammation to promote its therapeutic properties.	IL6, TNF-*α* (ELISA), NF-*κB*	The injection of 0.3 mM and 1 mM crocin resulted in a considerable decrease in the production of IL6 and TNF-*α*. Decreased modulation of NF-*κB* signaling Crocin has potential beneficial effects against skin aging.
3.	Aslani, M.R. *et al*. (2023)[34]	Randomized controlled trial	57 male patients with COPD	The experimental and control groups were administered 15 mg of Crocina™ tablets twice daily, along with a placebo, for 12 weeks. The participants ingested crocin or placebo tablets twice a day, in the morning and evening, alongside their main meals (breakfast and supper) for 12 weeks.	IL-6, TNF-*α* (ELISA from serum)	The crocin group did not experience any notable changes, whereas the placebo group had a considerable rise in blood IL-6 level following the intervention. There was no significant difference in the levels of TNF-*α* between the placebo group and the crocin group after the intervention.
4.	Shoyama, Y. *et al*. (2015)[35]	*In vivo*	Mice were administered azoximitan (AOM) and dextran sulfate sodium, which are used as promotional agents for induction of colorectal lesions.	Crocin at 100 ppm and 200 ppm	NF-*κB* (immunohisto chemistry of the colon) IL-6, TNF-*α*, NF-*κB* (RT-PCR)	Treatment with crocin at concentrations of 100 ppm and 200 ppm dramatically reduced immunohistochemistry scores for NF-kB. IL-6, TNF-*α*, NF-*κB* reduced

5.	Teng, S. *et al*. (2021)[36]	*In vivo*	60 C57BL/6J mice (male, 7–8 weeks old, weight 22–25 g) 20 ApcMinC/Gpt mice	The rats were allocated into five groups using randomization. Group I: model rats + saline. Group II: positive control rats + sulfasalazine 0.6 g/kg (SASP). Group III: rats + crocin 10 mg/kg. Group IV: rats + crocin 30 mg/kg (n=12) crocin once daily for 3 weeks. Group V: control. Crocin was administered orally for 3 weeks. The rats were separated into two groups: control + saline and crocin 30 mg/kg orally for 8 weeks.	IL-6, TNF-*α* (ELISA from serum and colon tissue) NF-*κB* (Western blot of colon tissue)	IL-6, and TNF-*α* were reduced after treatment. NF-*κB* expression decreased.

Table 3: Research of the potential role of crocin in modulating TNF-*α* and IL-6 levels in various diseases

No.	Reference	Research Design	Research Subject	Intervention	Variable	Results and Conclusions
1.	Liu, W. *et al*. (2018)[32]	Experimental *in vivo*	25 Rats CIA (Collagen induced arthritis)	The experimental rats were assigned to five groups using random allocation: saline control, CIA control, CIA + crocin 10 mg/kg rats, CIA + crocin 20 mg/kg rats; CIA + crocin 40 mg/kg rats.	TNF-*α*, IL-6 (ELISA from serum)	Serum levels of pro-inflammatory cytokines in CIA mice reduced to diverse extents when they were administered different dosages of crocin. The cohort of CIA mice who were administered the most potent dosage (40 mg/kg) of crocin demonstrated the least amount of pro-inflammatory cytokines in their serum, as compared to the other cohorts.

Shoyama *et al*.[35] stated that the administration of crocin at doses of 100 ppm and 200 ppm significantly suppressed immunohistochemical scores for NF-*κB*; in addition, IL-6, TNF-*α*, NF-*κB* were also reduced. The ability of crocin to decrease chemical-induced colitis and related colon cancer in mice by blocking inflammatory cytokines indicates its potential. These findings propose saffron and crocin as effective preventive agents for colitis and inflammation-associated colon cancer.[35]

Teng *et al*.[36] conducted an *in vivo* investigation using 60 C57BL/6J mice (male, 7–8 weeks old, 22–25 gr) and 20 ApcMinC/Gpt mice. The study found that crocin treatment resulted in a decrease in IL-6 and TNF-*α* levels, as well as a reduction in NF-*κB* expression. Crocin's anti-inflammatory properties were validated by its regulation of cytokine activity, particularly interleukins, via the modulation of NF-*κB* signaling. It also caused a significant decrease in interleukin and TNF-α levels in the bloodstream and colon tissue, suggesting its effects are mediated through the NF-*κB* pathway.[36]

The findings across the reviewed studies collectively underscore the potential of saffron and its active compounds, such as crocin and crocetin, in modulating pro-inflammatory cytokines, particularly TNF-α and IL-6. These compounds exhibit significant antioxidant and anti-inflammatory properties through mechanisms involving NF-*κB* inhibition and PPAR-γ activation, as well as the suppression of cyclooxygenase and other pro-inflammatory pathways. [18,21,23] The ability of saffron derivatives to neutralize free radicals further supports their role in mitigating inflammation in various chronic diseases, including rheumatoid arthritis, type 2 diabetes mellitus (T2DM), and neurodegenerative conditions. For example, studies like those of Rathore *et al*. and Samarghandian *et al*. demonstrated dose-dependent reductions in TNF-α and IL-6, suggesting the importance of precise dosage in therapeutic applications.[22,27] However, the inconsistent results in certain studies, such as Shahbazian *et al*.'s trial on T2DM patients, highlight the complexity of translating preclinical success into clinical efficacy, potentially due to variations in treatment regimens, population characteristics, or underlying disease mechanisms.[21]

Despite the promising results, the variability in study designs, such as differences in dosages, treatment durations, and disease models, introduces challenges in drawing definitive conclusions. Some studies, like those by Mobasseri *et al*. and Xiao *et al*., demonstrated robust anti-inflammatory effects with significant reductions in cytokine levels, while others, such as Hamidi *et al*., showed only moderate effects without reaching statistical significance.[23,26,28] These discrepancies may be attributed to differences in saffron formulation, bioavailability, and individual patient factors, such as disease stage or genetic predispositions. Additionally, variations in study populations—ranging from animal models to small-scale human trials—limit the generalizability of findings. To address these limitations, future research should focus on large-scale, multicenter randomized controlled trials (RCTs) with standardized protocols to better evaluate the therapeutic potential of saffron and its components.

Nevertheless, the overarching evidence positions saffron as a promising candidate for complementary therapies in managing inflammation-related conditions. Its ability to target key inflammatory mediators, alongside its favorable safety profile in most studies, offers potential advantages over conventional anti-inflammatory drugs, which often come with significant side effects. Future investigations should prioritize optimizing dosages and delivery methods to enhance the bioavailability of saffron's active compounds. Moreover, exploring synergistic effects with existing therapies could expand its applicability in clinical settings. Longitudinal studies are also crucial to assess the long-term safety and efficacy of saffron supplementation. By bridging the gap between preclinical insights and clinical implementation, saffron has the potential to become a valuable tool in combating chronic inflammation and its associated diseases. A summary of the effects of saffron extracts, crocin, and crocetin in reducing TNF-*α* and IL-6 levels is shown in Figure 1.

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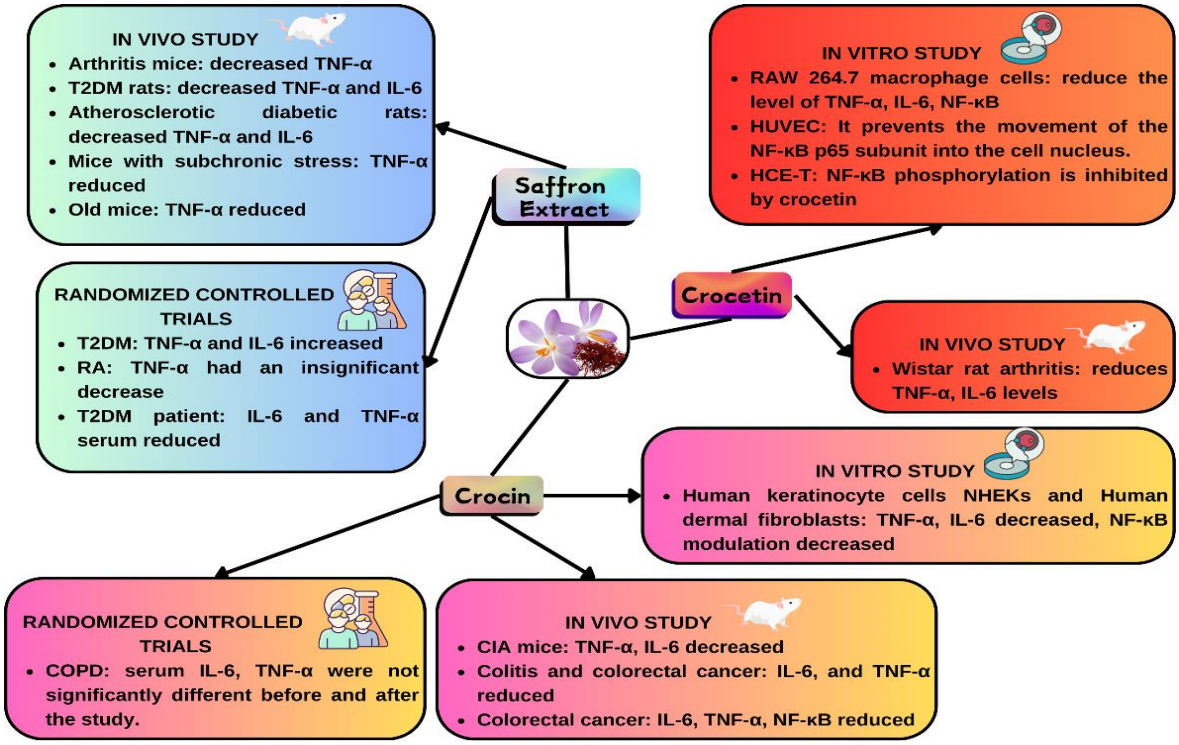

Figure 1: The Potential Role of Saffron Extract, Crocin, and Crocetin on Modulating TNF-*α* and IL-6 Levels in Various Diseases

Conclusion

This literature review indicates that saffron (*Crocus sativus*) and its constituents (crocin, crocetin) have the ability to decrease and control the levels of pro-inflammatory cytokines such as TNF-*α*, IL-6, although there are still some studies that state that the effect of saffron is not significant enough in reducing pro-inflammatory cytokines levels. *In vivo* and *in vitro* studies show that saffron and its components, including crocin, crocetin, and safranal, primarily exert their therapeutic effects by inhibiting inflammatory processes and scavenging free radicals. Nevertheless, RCT studies are still required to confirm the inconsistency of the results regarding saffron's potential role in modulating TNF-*α* and IL-6 levels in humans.

Looking forward, saffron and its derivatives have the potential to become significant tools in the management of inflammatory conditions. Beyond their direct anti-inflammatory effects, future studies could explore their potential synergistic interactions with existing treatments, such as NSAIDs or biologics, to enhance therapeutic efficacy while reducing side effects. Advances in drug delivery systems, including nanotechnology, could also improve the bioavailability of saffron's active compounds, ensuring more consistent therapeutic outcomes. Moreover, as interest grows in natural remedies, saffron could be integrated into personalized medicine approaches, where its anti-inflammatory effects are tailored to individual patient profiles.

In conclusion, while current evidence underscores the promise of saffron as a complementary or alternative treatment for inflammation-related conditions, further research is essential to fully understand its mechanisms and optimize its use. By addressing these gaps, saffron may emerge as a versatile and effective natural remedy, offering new possibilities for managing chronic inflammatory diseases and improving patient outcomes.

Conflict of interest

The authors declared no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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