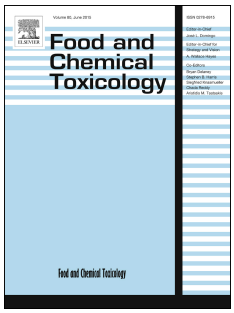
Journal Pre-proof



Crocin: A fighter against inflammation and pain

Mahmoud Hashemzaei, Charalampos Mamoulakis, Konstantinos Tsarouhas, George Georgiadis, George Lazopoulos, Aristidis Tsatsakis, Elham Shojaei Asrami, Ramin Rezaee

PII: S0278-6915(20)30411-7

DOI: https://doi.org/10.1016/j.fct.2020.111521

Reference: FCT 111521

To appear in: *Food and Chemical Toxicology*

Received Date: 11 May 2020

Revised Date: 7 June 2020

Accepted Date: 8 June 2020

Please cite this article as: Hashemzaei, M., Mamoulakis, C., Tsarouhas, K., Georgiadis, G., Lazopoulos, G., Tsatsakis, A., Asrami, E.S., Rezaee, R., Crocin: A fighter against inflammation and pain, *Food and Chemical Toxicology*, https://doi.org/10.1016/j.fct.2020.111521.

Crocin: A fighter against inflammation and pain

Mahmoud Hashemzaei[1#], Charalampos Mamoulakis[2], Konstantinos Tsarouhas[3#], George Georgiadis[2], George Lazopoulos,[4] Aristidis Tsatsakis[5], Elham Shojaei Asrami[6], Ramin Rezaee[6,7*]

1 Department of Pharmacodynamics and Toxicology, School of Pharmacy, Zabol University of Medical Sciences, Shahid Rajaii Street, Zabol, Iran

2 Department of Urology, University General Hospital of Heraklion, University of Crete, Medical School, Heraklion, Crete, Greece

3 Department of Cardiology, University Hospital of Larissa, Larissa, 41110, Greece

4Department of Cardiothoracic Surgery, University General Hospital of Heraklion, University of Crete, Medical School, Heraklion, Crete, Greece

5 Centre of Toxicology Science and Research, University of Crete, School of Medicine, Crete, Greece

6 Clinical Research Unit, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

7 Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

# Equal contribution as the first authors.

* Corresponding author: Ramin Rezaee

Email: raminrezaee1983@gmail.com AND rezaeera@mums.ac.ir

Abstract

Crocin, a water-soluble carotenoid, is known as a pharmacologically active compound, particularly for its potent anti-oxidant activity. The present work provides a comprehensive review of the available literature concerning the anti-inflammatory properties of crocin in various organs/systems as well as its anti-nociceptive effects. PubMed, Scopus, and Web of Science electronic databases were systematically searched up to 28 March 2020 to detect all relevant preclinical and human studies in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement. In total, 104 studies were included for qualitative synthesis. This systematic search and review indicated that crocin not only combats reactive oxygen species production and suppresses pro-inflammatory cytokines secretion but also alleviates inflammation in various organs (e.g. the lung, heart, brain, and kidney), in a series of animal models and *in vitro* experiments, via regulating mainly NF-κB pathway and NF-κBp65 translocation to the cell nucleus. In this context, modulation of PI3K/Akt appears to be a favorable crocin target contributing to NF-κB pathway inhibition. Even though data is limited in humans with only one clinically relevant study retrieved, the results of preclinical studies regarding anti-inflammatory/anti-nociceptive effects of crocin are promising and warrant further testing in clinical settings.

Crocin: A fighter against inflammation and pain

Mahmoud Hashemzaei[1#], Charalampos Mamoulakis[2], Konstantinos Tsarouhas[3#], George Georgiadis[2], George Lazopoulos,[4] Aristidis Tsatsakis[5], Elham Shojaei Asrami[6], Ramin Rezaee[6,7*]

1 Department of Pharmacodynamics and Toxicology, School of Pharmacy, Zabol University of Medical Sciences, Shahid Rajaii Street, Zabol, Iran

2 Department of Urology, University General Hospital of Heraklion, University of Crete, Medical School, Heraklion, Crete, Greece

3 Department of Cardiology, University Hospital of Larissa, Larissa, 41110, Greece

4 Department of Cardiothoracic Surgery, University General Hospital of Heraklion, University of Crete, Medical School, Heraklion, Crete, Greece

5 Centre of Toxicology Science and Research, University of Crete, School of Medicine, Crete, Greece

6 Clinical Research Unit, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

7 Neurogenic Inflammation Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

# Equal contribution as the first authors.

* Corresponding author: Ramin Rezaee

Email: raminrezaee1983@gmail.com AND rezaeera@mums.ac.ir

Highlights:

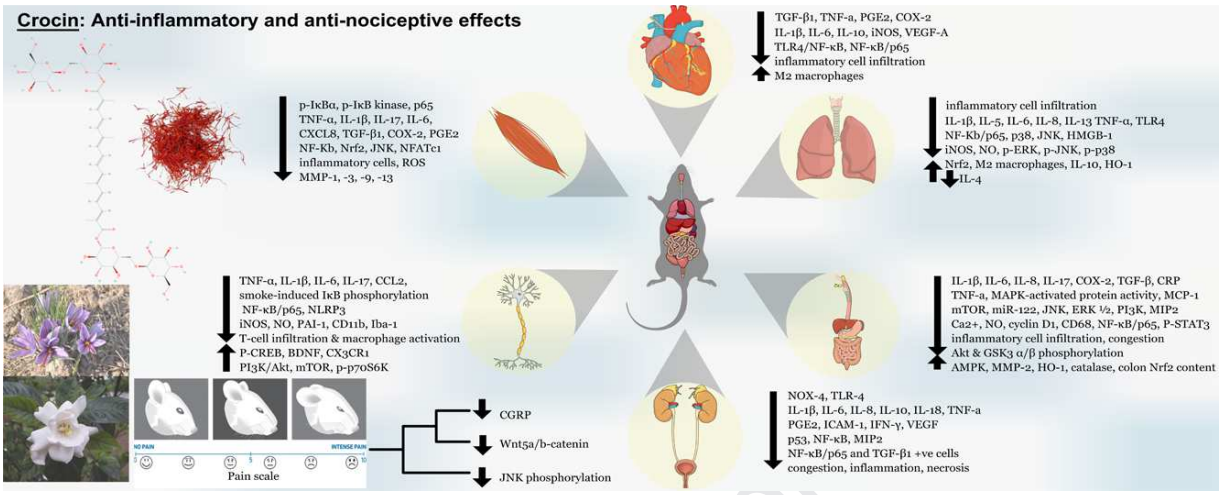
- Crocin anti-inflammatory function involves suppression of MAPK and NF-kB, but activation of Nrf2.
- Crocin suppresses production/secretion of TNF-α, IL-6, IL-10, IFN-γ and IL-1β (*in vitro* and *in vivo*) and reduces TNF-α and IL-17 in humans.
- Suppression of MAPK and Wnt5α/β-catenin signaling mediates crocin pain killing effects.
- Even though data is limited in humans, the results of preclinical studies regarding anti-inflammatory/anti-nociceptive effects of crocin are promising and warrant further testing in clinical settings. **Abbreviations:**
- 6-OHDA: 6-hydroxydopamine
- AA: Acetic acid
- ALP: alkaline phosphatase
- AMPK: 5'-adenosine monophosphate (AMP)-activated protein kinase
- AP: acute pancreatitis
- ARDS: Acute respiratory distress syndrome
- BDNF: Brain derived neurotrophic factor
- BPA: Bisphenol A
- CAMK4: Ca2+/calmodulin dependent protein kinase 4
- CAT: catalase
- CCL2: CC-chemokine ligand 2
- CCl4: carbon tetrachloride
- CGRP: calcitonin gene related peptide
- COPD: Chronic obstructive pulmonary disease

- COX: cyclooxygenase
- CREB: cAMP response element binding protein
- CRP: C Reactive Protein
- DSS: dextran sodium sulfate
- ERK: extracellular signal-regulated kinases
- FOXO3: forkhead box O3
- GPx: glutathione peroxidase
- GSK3α/β: glycogen synthase kinase 3α/β
- GST: glutathione S-transferase
- H&E: hematoxylin and eosin
- HAases: hyaluronidases
- HMGB1: High mobility group box protein 1
- HO-1: Heme oxygenase-1
- HSP: Heat shock protein
- ICAM: Intercellular adhesion molecules
- IFN: Interferon
- IgE: Immunoglobulin E
- IL: Interleukin
- iNOS: Inducible nitric oxide synthase
- ip: intraperitoneal
- IR: Ischemia/Reperfusion
- JNK: c-Jun N-terminal Kinase
- LPS: Lipopolysaccharide
- MAPK: Mitogen-activated protein kinase
- MAPKAPK: MAPK-activated protein kinases

- MCP: monocyte chemotactic protein
- MCP-1: monocyte chemoattractant protein-1
- MDA: Malondialdehyde
- MIP: macrophage inflammatory proteins
- MMP: Matrix metalloprotease
- MS: multiple sclerosis
- mTOR: mammalian target of rapamycin
- NAFLD: Non-alcoholic fatty liver disease
- NF-κB : Nuclear Factor-kappa B
- NLRP3: NLR family pyrin domain-containing protein 3
- NOX: NADPH oxidase
- Nrf2: nuclear factor erythroid derived 2 like 2
- OVA: ovalbumin
- PAI-1: plasminogen activator inhibitor type 1
- PDGFB: platelet-derived growth factor
- PGE2: Prostaglandin E2
- PI3K: Phosphoinositide 3-kinase
- PKC: Protein kinase C
- PRAS40: proline-rich Akt substrate of 40 kDa
- RANKL: receptor activator of nuclear factor-kappaB ligand
- ROS: Reactive oxygen species
- SOD: superoxide dismutase
- STAT6: signal transducer and activator of transcription 6
- STZ: Streptozotocin
- TBI: traumatic brain injury

- TGF: Transforming growth factor
- Th: T helper
- TLR4: Toll-Like Receptor 4
- TNF: Tumor necrosis factor
- UC: Ulcerative colitis
- URIRI: Unilateral renal ischemia reperfusion injury
- VEGF: vascular endothelial growth factor
- VEGF: vascular endothelial growth factor

Graphical Abstract



Abstract

Crocin, a water-soluble carotenoid, is known as a pharmacologically active compound, particularly for its potent anti-oxidant activity. The present work provides a comprehensive review of the available literature concerning the anti-inflammatory properties of crocin in various organs/systems as well as its anti-nociceptive effects. PubMed, Scopus, and Web of Science electronic databases were systematically searched up to 28 March 2020 to detect all relevant preclinical and human studies in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement. In total, 104 studies were included for qualitative synthesis. This systematic search and review indicated that crocin not only combats reactive oxygen species production and suppresses pro-inflammatory cytokines secretion but also alleviates inflammation in various organs (e.g. the lung, heart, brain, and kidney), in a series of animal models and *in vitro* experiments, via regulating mainly NF-κB pathway and NF-κBp65 translocation to the cell nucleus. In this context, modulation of PI3K/Akt appears to be a favorable crocin target contributing to NF-κB pathway inhibition. Even though data is limited in humans with only one clinically relevant study retrieved, the results of preclinical studies regarding anti-inflammatory/anti-nociceptive effects of crocin are promising and warrant further testing in clinical settings.

Key words: crocin; natural products; herbal medicine; pain; inflammation.

Introduction

Crocin, a bioactive natural product isolated from *Gardenia jasminoides* Ellis and *Crocus sativus* (saffron), is a water-soluble carotenoid that contributes to the red color of saffron (Huang, Xu et al. 2019, Kalantar, Kalantari et al. 2019). From a chemical point of view, crocin is a diester consisted of the disaccharide gentiobiose and the dicarboxylic acid crocetin (Alavizadeh and Hosseinzadeh 2014). These phytoconstituents are radical-scavengers, particularly against superoxide anions (Erben-Russ, Michel et al. 1987, Rezaee and Hosseinzadeh 2013, Kocaman, Altinoz et al. 2019). Besides cytotoxic (Rezaee, Mahmoudi et al. 2013, Rezaee, Jamialahmadi et al. 2014), anti-hypertensive (Hashemzaei, Rezaee et al.), antidepressant, anti-atherosclerotic, anti-platelet aggregation, and nephron-protective properties (Korani, Korani et al. 2019), crocin possesses marked anti-oxidant activities as well as anti-inflammatory properties (Hatziagapiou, Kakouri et al. 2019, Suh, Chon et al. 2019).

The well-known antioxidant properties of crocin were shown to be mediated, at least in part, through modulation of glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT), and superoxide dismutase (SOD)(Korani, Korani et al. 2019). Reactive oxygen species (ROS) are regularly produced during metabolic activities of the cell; however, under uncontrolled conditions, they affect redox balance subsequently disturbing stress responses and apoptotic signaling pathways with deleterious consequences (Chiu and Dawes 2012, Rezaee, Behravan et al. 2014). Besides, inflammatory diseases were shown to be associated with ROS production/release (Kavasi, Berdiaki et al. 2017, Korani, Korani et al. 2019, Tsoukalas, Fragoulakis et al. 2019, Zalar, Pop et al. 2019). In this regard, oxidative-induced changes in proteins were reported to affect their structure and activity or render them active mediators in

inflammation development (Ungurianu, Margină et al. 2017, Hatziagapiou, Kakouri et al. 2019, Neagu, Constantin et al. 2019).

Inflammation is an extremely essential protective function of an organism and various patterns have been described for different diseases since ancient times (Kuprash and Nedospasov 2016). Modern life which exposes the individuals to a multitude of stimuli from different sources, even at low doses, is a trigger of oxidative stress, inflammation and chronic diseases in long term (Tsatsakis, Kouretas et al. 2017, Docea, Gofita et al. 2018, Fountoucidou, Veskoukis et al. 2019, Tsatsakis, Tyshko et al. 2019, Tsatsakis, Docea et al. 2019). Type 1 T helper (Th1), Th2, and Th17 as the key parts of the classic immune responses, exert their characteristic functions via particular cytokines. Th1 responses involve interferon-gamma (IFN-γ) and interleukin (IL)-12 cytokines, and induce cellular immune responses. Th2 and Th17 respectively produce allergic (through IL-4, IL-5 and IL-13), and neutrophil-mediated (through IL-17) reactions. Cytokines that act as immunosuppressants are IL-10, and transforming growth factor beta (TGF-β) (Korani, Korani et al. 2019).

Nuclear factor-κB (NF-κB) is considered a main player in various inflammatory processes (Schuliga 2015) and when it interacts with a particular DNA sequence of TGF-β1 gene, it induces the expression of TGF-β1 gene (Bowie and O'Neill 2000). Mechanistically, as a reaction to an external stimuli, IκB kinase activity leads to IκBα ubiquitination and proteasomal destruction, and subsequently, NF-κB translocation to the nucleus (Jiang, Yi et al. 2015). Receptor activator of NF-κB ligand (RANKL) triggers classical NF-κB signaling by inducing IκBα kinase (IKK), and phosphorylation-dependent IκBα degradation, followed by translocation of p65 to the nucleus (Li and Verma 2002). It is postulated that ROS mediate NF-κB-activation,

as it was suppressed by several antioxidants or upregulated antioxidant enzymes (Li and KARIN 1999).

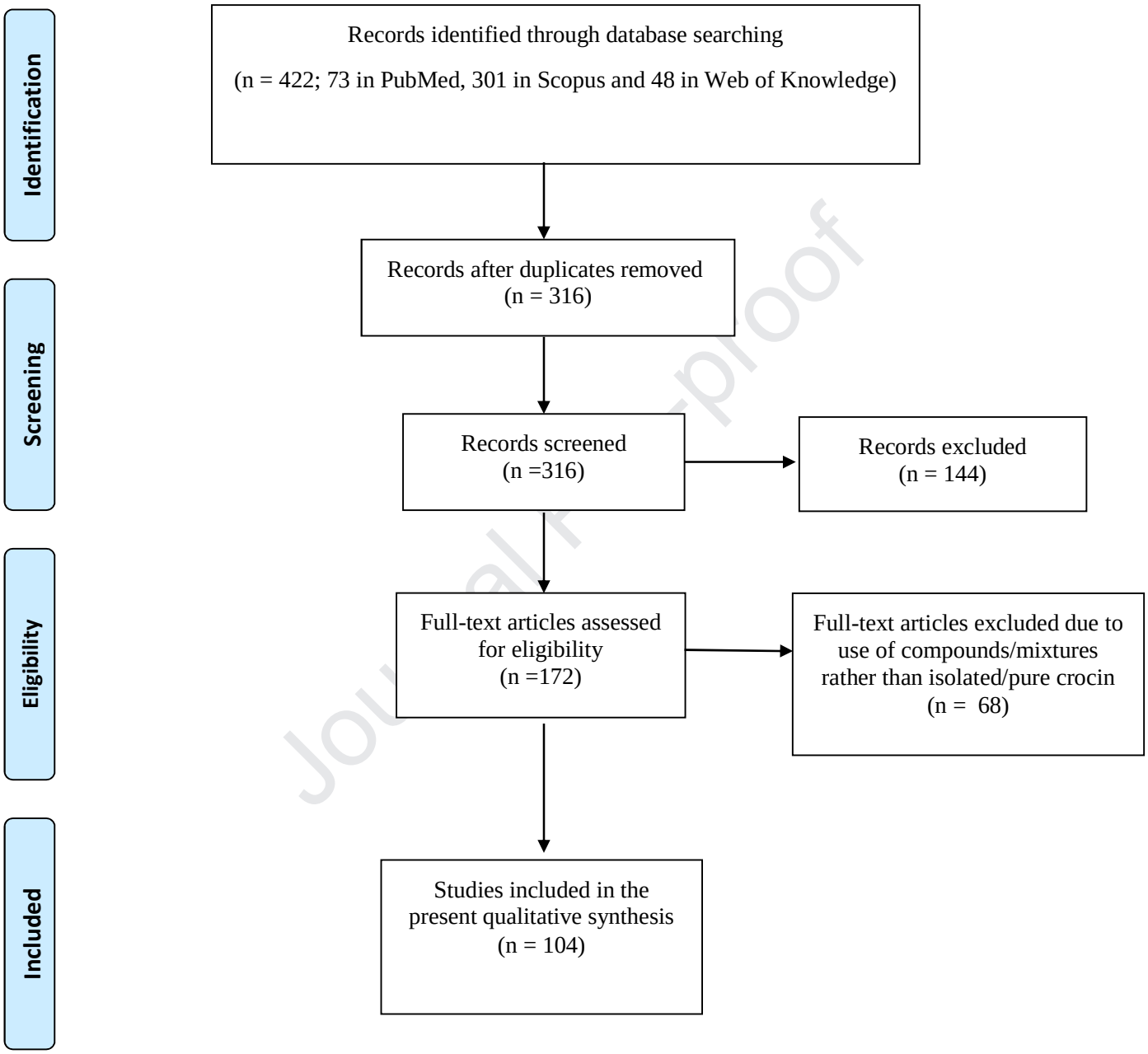
The aim of this paper is to provide a comprehensive review of the available literature on the potential anti-inflammatory properties of crocin in various organs/systems and its anti-nociceptive effects, focusing on the underlying mechanisms.

Methods

PubMed, Scopus, and Web of Science electronic databases were systematically searched (28 March 2020) to detect all original research studies, according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement (Moher, Liberati et al. 2010). The specific literature search strategy used was: (crocin AND ("*pain*" OR "*inflammation*" OR "*inflam*" OR "ache" OR "*algia*")). The reference lists of selected studies were screened for other potentially eligible studies. After excluding duplicates, citations in abstract form and non-English citations, the titles/abstracts of full papers were screened for relevance. All types of citations other than original research studies (e.g. review articles) and those reporting the effects of extracts (i.e. not pure crocin) were excluded. Two review authors (M.H., R.R. and E.S.A) independently assessed the title and the abstract content (or both) of every record retrieved to decide which studies should be further evaluated and extracted all data. Disagreements were resolved through consensus or by consultation with a third author (K.T.). A final draft of the manuscript was prepared after several revisions (C.M., K.T., G.G., G.L. A.T. E.S.A., and R.R.) and approved by all authors. In total, 104 studies (103 preclinical studies and oneclinical trial) were considered for qualitative synthesis (Figure 1).

192 **Figure 1.** PRISMA flowchart showing the process of article selection.
193

194



196 **Anti-inflammatory effects in the respiratory system**

197 Crocin was administered to mice that were exposed to cigarette smoke for induction of
198 chronic obstructive pulmonary disease (COPD); results showed that crocin could reduce
199 inflammatory cells number and lung IL-1β, IL-6 and TNF-α levels; however, IGF-1 (insulin-like
200 growth factor 1), a PI3K/Akt activator, neutralized crocin's effect suggesting that crocin exerts
201 its anti-inflammatory effects through modulation of PI3K/Akt-mediated inflammatory pathways
202 (Xie, He et al. 2019).

203 In rats with COPD, crocin up-regulated mRNA levels of Nrf2 (erythroid-derived 2 like
204 protein 2) via induction of mRNA expression of PKC (protein kinase C), PI3K
205 (phosphoinositide-3-kinase) and MAPK (mitogen-activated protein kinases; ERK2), as its
206 upstream activators (Dianat, Radan et al. 2018). Consistently, crocin protected cigarette smoke
207 extracts-exposed alveolar epithelial cells (A549) via activation of Nrf2 and boosting anti-oxidant
208 system (Radan, Dianat et al. 2019).

209 Moreover, human bronchial epithelial cells pre-treated with crocin showed lower
210 concentrations of TNF-α, IL-8, IL-6 and IL-1β as well as suppressed NF-κB p65 expression and
211 nuclear translocation induced by *Aspergillus fumigatus* (Du, Chi et al. 2018).

212 In an *in vitro* study in RAW264.7 cells, it was observed that crocin induced IL-4 and IL-
213 10 which are considered anti-inflammatory mediators but diminished TNF-α and IL-6 that are
214 regarded as pro-inflammatory components. Anti-inflammatory activity of crocin was mediated
215 through favoring M2 macrophage differentiation through suppressing p38 and JNK pathways
216 (Zhu, Yang et al. 2019). Also, in RAW264.7 cells incubated with lipopolysaccharide (LPS),
217 crocin increased heme oxygenase-1 (HO-1) expression which resulted in an anti-inflammatory
218 response; further, crocin suppressed iNOS and NO by down-regulation of NF-κB. Interestingly,

it was observed that Ca^{2+}/calmodulin dependent protein kinase 4 (CAMK4) inhibition, abrogated HO-1 expression, Nrf2 activation, and Akt phosphorylation; thus, authors concluded that iNOS suppression was mediated through increment of HO-1 expression as a result of Ca^{2+}/calmodulin-CAMK4-PI3K/Akt-Nrf2 signaling activation (Kim, Park et al. 2014).

In rats treated with intratracheal bleomycin, crocin markedly diminished total inflammatory cells, lymphocytes and neutrophils number in bronchoalveolar lavage fluid (BALF) and considerably decreased scores of lung's inflammatory lesions; also, crocin suppressed the lung levels of IL-10 and TLR4 (toll-like receptor 4), the latter is central in neutrophil accumulation and TNF-α expression, and when activated, induces TGF-β1 signaling and activates fibroblasts (Zaghloul, Said et al. 2019).

Yosri et al. treated the mice with ovalbumin (OVA) to produce a murine model of asthma that showed higher lung levels of TNF-α, IL-4, and IL-13 and elevated number of BALF inflammatory cells (Yosri, Elkashef et al. 2017). TNF-α is involved in commencement and development of tissue damage. In lungs, TNF-α stimulates neutrophil attachment to the capillaries and subsequent entrance to the alveolar space (Ware 2006, Giebelen, van Westerloo et al. 2007). Beside, via IL-4-mediated signaling, IL-13 induces signal transducer and activator of transcription 6 (STAT6), resulting in airway hyper-responsiveness (Grünig, Warnock et al. 1998, Wills-Karp, Luyimbazi et al. 1998). In this experiment, all OVA-induced alterations were restored by crocin (Yosri, Elkashef et al. 2017). Consistently, in murine asthma models, crocin alleviated airway inflammation via modifying IL-4/IL-13 or MAPK signaling (Xiong, Wang et al. 2015, Yosri, Elkashef et al. 2017).

Moreover, in BALB/c mice with allergic airway inflammation induced by OVA, crocin ameliorated airway inflammation and hyper-reactivity, diminished IL-4, IL-5, IL-13 and tryptase

in the BALF, reduced serum OVA-specific IgE, and repressed expression of major MAPKs namely, p-ERK, p-JNK and p-p38 in the lung (Xiong, Wang et al. 2015). LPS-induced increment of BALF IL-1β and TNF-α levels was down-regulated by crocin in mice with acute lung injury (Wang, Kuai et al. 2015). Using LPS-induced model of lung injury, it was also found that besides reduction of lung levels of phosphorylated p38, ERK, and JNK, crocin pretreatment suppressed HMGB-1 (high-mobility group box 1) which is a late inflammatory factor which induces intracellular NF-κB signaling pathway and worsens the injury (Zhang, Qi et al. 2020).

Main characteristics of the detected preclinical studies investigating the potential anti-inflammatory properties of crocin in the respiratory tract, are summarized in Table 1 (Kim, Park et al. 2014, Xiong, Wang et al. 2015, Yosri, Elkashef et al. 2017, Dianat, Radan et al. 2018, Du, Chi et al. 2018, Xie, He et al. 2019, Zaghloul, Said et al. 2019, Zhang, Qi et al. 2020).

Anti-inflammatory effects in the nervous system

In a rat model of Parkinson's disease induced by 6-hydroxydopamine (6-OHDA), crocin effects were examined and it was reported that crocin could reduce striatum levels of TNF□α (Shahidani, Rajaei et al. 2019). In rats with Parkinson's disease caused by exposure to rotenone, crocin activated PI3K/Akt, and augmented phospho-proline-rich Akt substrate 40 kDa (p-PRAS40), mTOR and p-p70S6K. Also, miRNA-7 and miRNA-221 expression was induced which led to Akt/mTOR activation (Salama, Abdel-Latif et al. 2020). Phosphorylation of PRAS40 leads to mTOR activation (Chong 2016). PI3K Activation can stimulate the activation of mTORC2 and further activation of p-Akt. mTOR activation results in activation of its downstream target p70S6K which contributes to cells survival (Laplante and Sabatini 2012).

Also, crocin effects on malathion-induced Parkinson-like symptoms, were documented by reduced striatum and hippocampal levels of TNF-α and IL-6 (Mohammadzadeh, Hosseinzadeh et al. 2018, Mohammadzadeh, Abnous et al. 2020).

In a study that examined crocin effects on inflammatory lung condition and depression as a comorbidity, NF-κB pathway involvement in the effects of crocin on cigarette smoke-induced COPD in mice, was evaluated; crocin treatment led to reduced levels of smoke-induced IκB phosphorylation and degradation, inhibition of translocation of NF-κBp65 to the nucleus and restoration of pro-inflammatory cytokines concentrations in the hippocampus. Among pathways that crocin can modulate, Xie et al. found that crocin could modify NF-κB by inhibiting PI3K/Akt-mediated signaling as application of IGF-1 neutralized crocin's effect (Xie, He et al. 2019).

Involvement of neuro-inflammation and oxidative stress in induction of depression and anxiety was documented by augmented IL-1β, IL-6, and TNF-α levels in patients (Dowlati, Herrmann et al. 2010). In this context, crocin-I effect was tested in chronic corticosterone-depressed mice; results showed that oral gavage of crocin-I, as a member of crocin family (comprised of crocin I-IV), reduced IL-1β expression and oxidative stress in mice hippocampus (Xiao, Xiong et al. 2019).

In a experiment that studied LPS-induced anxiety and depressive-like behaviors, LPS-treated BV2 microglia cells incubated with crocin showed reductions in TNF-α and IL-1β in comparison to the LPS only treated control cells; in parallel, researchers showed that NLR family pyrin domain-containing protein 3 (NLRP3) inflammasome that stimulates pro-caspase-1, which sequentially triggers IL-1β and IL-18 cleavage and secretion, was attenuated by crocin. NLRP3 mRNA expression is stimulated by NF-κB (Zhang, Previn et al. 2018). Moreover, in

BV-2 microglia stimulated by LPS, crocin inhibited gene expression of IL-6, CC-chemokine ligand 2 (CCL2), and iNOS and reduced NO production; however, authors concluded that suppression of NF-κB translocation to the nucleus was not involved in the anti-inflammatory effects (Yorgun, Rashid et al. 2017).

Also, crocin protective activity against methamphetamine-induced neuro-inflammation was evidenced by reduced hippocampal level of TNF-α (Shafahi, Vaezi et al. 2018). Similarly, crocin lowered hippocampal TNF-α and IL-1β concentrations in methamphetamine-treated rats (Mozaffari, Yasuj et al. 2019).

Methylphenidate is a psycho□stimulant drug with marked addictive potential; this agent is being used for treatment of attention/deficit hyperactivity disorder (ADHD) in children (Morton and Stockton 2000, Leonard, McCartan et al. 2004). In methylphenidate-treated rats, crocin administration decreased hippocampal IL□1β and TNF□α concentrations, but induced phosphorylated CREB (P□CREB) and brain-derived neurotrophic factor (BDNF) (Ebrahimzadeh, Moghadam et al. 2019). P□CREB (that is activated CREB), governs several genes specially BDNF which has a key role in regeneration, development, survival, excitability, addiction, depression, and cognition. Importantly, disruption of CREB pathway was found to be followed by oxidative stress, apoptosis, and neurodegeneration (Mayr and Montminy 2001). BDNF protective effects on brain cells and neurons are mediated through its receptor, TrkB (Martinowich, Hattori et al. 2003, Carlezon Jr, Duman et al. 2005).

Moreover, treatment of BV-2 cells with crocin, resulted in decrement of LPS-stimulated productions of TNF-α and IL-1β and repression of LPS-elicited NF-κB activation (Nam, Park et al. 2010). Similarly, crocin could reduce serum concentrations of IL-6, IL-1β, and TNF-α in a rat model of Alzheimer's disease (Sadoughi 2019).

Furthermore, crocin diminished levels of plasminogen activator inhibitor type 1 (PAI-1), which is linked to inflammatory conditions like septic shock, in brain and liver samples from LPS-treated rats (Tsantarliotou, Lavrentiadou et al. 2019).

Considering such evidence, it was suggested that crocin, by affecting CREB/BDNF and Akt/GSK pathways, might be potentially beneficial for prevention/ treatment of alcohol-induced neurodegeneration and neurobehavioral outcomes (Motaghinejad, Safari et al. 2019). Akt hyperactivity was shown to be involved in various common malignancies and impairment of Akt signaling was shown in neurodegenerative conditions like Alzheimer's and Huntington disease. Moreover, association between impaired Akt/GSK3 signaling and schizophrenia was reported. Glycogen Synthase Kinase 3 (GSK3) is a target for Akt1 and it was reported to be modulated in schizophrenia as lower levels of GSK3α proteins in lymphocytes from patients with schizophrenia (Yang, Yu et al. 1995) and involvement of GSK3β in the disease (Emamian 2012), were shown. GSK3 is extensively found among eukaryotes and more than a hundred proteins were reported to be specifically GSK3 substrates. Mammalian GSK3 is classified into GSK3α and GSK3β (Hermida, Kumar et al. 2017).

In an experimental autoimmune encephalomyelitis (EAE), crocin could down-regulate the expression of inflammatory genes in the spinal cord and suppress infiltration of T cells and activation of macrophages (Deslauriers, Afkhami-Goli et al. 2011). In another study, also crocin treatment reduced spinal cords TNF- α level, suggesting a protective property for the compound against spinal cord injury (Terraf, Kouhsari et al. 2017).

Stimulated microglial cells can induce overgeneration and secretion of factors such as TNF-α, IL-1β, IL-6, NO, and ROS that contribute to inflammation/cytotoxicity (Graeber and

Streit 2010). Thus, microglial cells over-activation can be considered an initial step towards retinopathy and degeneration of the optic nerve (Bosco, Steele et al. 2011, Bosco, Romero et al. 2015); findings of Yang et al. (Yang, Huo et al. 2017) showed the potential efficacy of crocin against diabetic retinopathy. Considering the deleterious effect of microglial activation on retinal ganglion cells (RGCs) and its potential involvement in glaucoma development, crocin inhibitory effects on primary RGCs and LPS-stimulated BV-2 cells, were examined. Results showed lower levels of LPS-induced microglial markers (CD11b and Iba-1) and iNOS, cyclooxygenase (COX)-2, IL-1β, and TNF-α as pro-inflammatory factors, when cells were pretreated with crocin but it improved protein levels of PI3K and p-Akt; also, in BV-2 cells, a significant increment of CX3CR1 (membrane-bound fractalkine receptor mainly expressed on microglia) expression caused through repression of NF-κB/Yin Yang 1 (YY1), was found. Crocin-induced inhibition of microglial over-activation might potentially suggest it as a protective agent against neurodegeneration in glaucoma (Lv, Huo et al. 2016).

The effects of crocin in reducing DNA damage, inflammation, and oxidative stress in patients with multiple sclerosis (MS) were evaluated in a double-blind, randomized placebo-controlled trial (Ghiasian, Khamisabadi et al. 2019). A total of 40 patients were randomly assigned 1:1 into an intervention and control group receiving two crocin capsules (30 mg/day) or placebo per day, respectively, for 28 days. Based on the results, TNFα, and IL-17 were markedly decreased in the serum of crocin-treated subjects compared to the placebo group.

Main features of the detected preclinical investigations dealing with potential anti-inflammatory activities of crocin in the nervous system, are summarized in Table 2 (Karami, Bathaie et al. 2013, Wang, Zhang et al. 2015, Heidari, Mehri et al. 2017, Yang, Huo et al. 2017, Yorgun, Rashid et al. 2017, Mohammadzadeh, Hosseinzadeh et al. 2018, Shafahi, Vaezi et al. 2018,

Zhang, Previn et al. 2018, Ebrahimzadeh, Moghadam et al. 2019, Mozaffari, Yasuj et al. 2019, Sadoughi 2019, Shahidani, Rajaei et al. 2019, Tsantarliotou, Lavrentiadou et al. 2019, Xiao, Xiong et al. 2019, Xie, He et al. 2019, Salama, Abdel-Latif et al. 2020).

Anti-inflammatory effects in the gastrointestinal system

Chronic liver inflammation results in liver cirrhosis and fibrosis. Liver cirrhosis is ranked the 12th cause of death in the US. Viral infection, alcoholic liver disease, nonalcoholic steatohepatitis, and autoimmune disorders are chronic pathologic conditions that may lead to the above-noted hepatic issues (Koyama and Brenner 2017).

In mice with hepatic injury, crocin down-regulated IL-1β and TNF-α levels (Kalantar, Kalantari et al. 2019). In mice with cyclophosphamide-induced hepatotoxicity, crocin treatment led to decrement of serum concentrations of IL-1β and IL-6 as well as TNF-α, which have critical roles in oxidative stress-induced liver inflammation (Jnaneshwari, Hemshekhar et al. 2013, Hassani, Rezaee et al. 2015). In rats with carbon tetrachloride (CCl_4)-induced hepatotoxicity, crocin ameliorated the levels of IL-6 and TNF- α, normalized liver histology, suppressed caspase 3 and oxidative stress and modulated hepatic metabolizing enzymes toward removal of CCl_4 toxic metabolite of (Bahashwan, Hassan et al. 2015). In db/db mice with fatty liver and diabetic and obese conditions, crocin ameliorated hepatic inflammation along with induction of AMP-activated protein kinase (AMPK) but repression of mammalian target of rapamycin (mTOR) phosphorylation in the liver (Luo, Fang et al. 2019). Hepatic histological studies from acrylamide-exposed rats treated with crocin, showed significant amelioration of inflammatory cell infiltration and vascular congestion compared to non-treated animals (Gedik, Erdemli et al. 2017). In another study which employed a bisphenol A (BPA)-induced model of liver injury, crocin downregulated miR-122 and JNK transcript levels and ERK1/2 and MAPK-

activated protein kinases (MAPKAPK) phosphorylation and activity which were all increased by BPA; at the same time, crocin ameliorated BPA-induced morphological damages in the liver (Hassani, Mehri et al. 2017). miR-122 is a key player in the liver both under physiological and pathological conditions; also, it is regarded as a biomarker of liver conditions such as chronic hepatitis and non-alcoholic fatty-liver disease (Bandiera, Pfeffer et al. 2015). Moreover, JNK as a MAPK, governs important processes in the cell (ranging from gene expression and translation to cell proliferation/differentiation and death) (Cargnello and Roux 2011). MAPK signaling has a pivotal role in hepatic metabolism (Lawan, Zhang et al. 2015).

C Jun NH2-terminal kinase (JNK/SAPK), extracellular signal-regulated kinases 1 and 2 (ERK1/2), and p38 kinase, as mitogen-activated protein kinase (MAPKs), regulate cellular functions like gene expression, as well as cell proliferation and differentiation, survival and death (Cargnello and Roux 2011, Hashemzaei, Imen Shahidi et al. 2016, Hashemzaei, Delarami Far et al. 2017). Activated MAPKs are able to induce other kinase targets, and subsequent to translocation to the nucleus, they can induce pro-inflammatory genes expression (Saklatvala 2004).

Xenobiotic-induced cell death was shown to be mediated via ERK-JNK activation followed by increment of oxidative/inflammatory responses (Wang, Yen et al. 2008, Wu, Yen et al. 2012, Ki, Park et al. 2013). Moreover, MAPKAPK2 was shown to be involved in inflammation (Kotlyarov, Neininger et al. 1999) and upregulated in various cancers (Felix, Colleoni et al. 2009, Liu, Yang et al. 2012). In this regard, in H_2O_2-treated bovine aortic endothelium (BAE-1) cells, crocin ameliorated elevated phosphorylation and activation of p44/42 MAPK (ERK1/2), blocked ERK1/2 activation but activated JNK/SAPK (Rahiman, Akaberi et al. 2018). It should be noted that though it was widely shown that ERK1/2 activation

induces signals that promote survival, contradictory reports indicated that constant activation of ERK1/2 leads to cells death (Zhang and Liu 2002).

Crocin could also alleviate liver fibrosis in mice through reduction of NF-κB, COX-2, IL-1β and TNF-α expression and reverted TGF-β up-regulation as well as metalloproteinase (MMP)-2 down-regulation which are indicators of fibrosis pathogenesis (Algandaby 2018). Crocin also alleviated hepatic TNF-α and IL-6 levels in ethanol-intoxicated rats (Rezaee Khorasany, Razavi et al. 2020).

Considerable amount of evidence has shown the contribution of immunologic (i.e. Th1-, Th2- or Th17-mediated inflammatory response), microbiologic and genetic factors to inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC) development (Blumberg 2009). Especially, since gut is considered a barrier organ, inflammation is always of high importance in gastroenterology (Ribaldone, Pellicano et al. 2019) .

A study that treated a rat model of UC with crocin, showed that crocin was able to lower C reactive protein levels (CRP, which was found to be correlated with the disease magnitude) (Iwasa, Yamada et al. 2015). At the same time, crocin reduced colon TNF-α and Ca^{2+} levels, while increased colon HO-1 activity and colon Nrf2 content (Khodir, Said et al. 2019); suppression of Nrf2 signaling increases susceptibility towards either oxidative or electrophilic stress, resulting in tissue injury (Khor, Huang et al. 2006, Panieri, Buha et al. 2020). In another study on acetic acid-induced UC, crocin reduced TNF-α in colon samples and suppressed inflammatory cells infiltration (Faramarzpour, Tehrani et al. 2019). Similarly, administration of crocin to mice with UC caused by dextran sodium sulfate (DSS)-treatment, ameliorated histopathological features, increased catalase activity, reduced NO levels and lessened hs-CRP, a pro-inflammatory marker, concentrations in serum and colon samples. Also, crocin revered DSS-

induced cyclin D1 expression and GSK3α/β phosphorylation/inactivation (Rezaei, Avan et al. 2019). Cyclin D1 as a Wnt/β-catenin target gene, is a regulator of the G1 phase in cell cycle (Takahashi-Yanaga and Sasaguri 2008). In G1, cyclin D1 accumulates in the nuclei and in the S phase, moves to the cytoplasm (Baldin, Lukas et al. 1993). GSK3β phosphorylates nuclear cyclin D1 (Diehl, Cheng et al. 1998). It was documented that NF-κB stimulates cyclin D1 transcription (Hinz, Krappmann et al. 1999) which is in agreement with the presented data. Also, it was previously shown that activation of GSK3α/β promotes multiple inflammatory diseases all over the body (Jope, Yuskaitis et al. 2007). Also, effect of crocin (orally) on DSS-induced colitis□ associated colorectal cancer, was in female mice; based on hematoxylin and eosin (H&E) staining, inflammation score and pathological alterations were ameliorated by crocin. Also, crocin concentration□dependently suppressed PI3K expression and its downstream targets cyclin D1 and surviving, and inhibited Akt and GSK3α/β phosphorylation, indicating modulation of PI3K/Akt and Wnt pathway by the compound (Amerizadeh, Rezaei et al. 2018). Consistently, crocin beneficial effects against DSS-colitis were confirmed by another study in mice (Kawabata, Tung et al. 2012).

Crocin could also reduce inflammatory cells infiltration in the intestine of rats treated with CCl_4 (Cosgun, Erdemli et al. 2018). Crocin's protected against acute pancreatitis as documented by reduction of pro-inflammatory cytokines (IL-1β, IL-6, IL-17, and TNF-α) levels, repression of cytosolic and nuclear levels of NF-κB and upregulation of Nrf2 levels (Godugu, Pasari et al. 2019). IL-6, secreted by T-cells, induces immune responses which ultimately result in inflammation (Mofidi, Duff et al. 2006). Nrf2 possesses cytoprotective properties and regulates antioxidant proteins as it migrates to the nucleus under oxidative stress conditions and induces antioxidant genes expression (Loboda, Damulewicz et al. 2016). It was reported that

Nrf2 induction leads to higher levels of HO-1 activity; the anti-inflammatory function of HO-1 was evidenced by reduced levels of inflammatory mediators (Yalniz, Demirel et al. 2012). In ethanol-ulcerated rats, it was also shown that neutrophil infiltration is mainly induced by TNF-α which itself is an activator of NF-κB pathway (Hamaguchi, Watanabe et al. 2001).

Mounting evidence is showing the role of inflammatory pathways as major pathogenetic players in development of diabetes when risk factors such as western and sedentary lifestyle exists (Tsalamandris, Antonopoulos et al. 2019). Considerable body of data on protective effects of crocin against diabetes and metabolic syndrome, motivated us to discuss such effects with a focus on anti-inflammatory mechanisms.

In streptozocin (STZ)-diabetic rats, crocin diminished macrophages within the β-islets as confirmed by reduced CD68 expression (Samaha, Said et al. 2019). TNF-α plasma levels were reduced by crocin in mice with fructose-induced metabolic syndrome (El-Fawal, El Fayoumi et al. 2019); such effect was also observed in rats irradiated with γ-ray (Tawfik and Elkady 2019). In another study done in rats with metabolic syndrome induced by fructose (10%), crocin resulted in reduced serum TNF-α levels and inflammatory cells (El-Fawal, El Fayoumi et al. 2018). Besides, diabetes-induced increment of plasma concentrations of TNF-α and IL-1β and pancreas concentration of TNF-α, was decreased by crocin, *in vivo* (HAZMAN, Aksoy et al. 2016).

Chemopreventive function of crocin was investigated in rats with hepatocellular carcinoma (HCC) and in HepG2 cells; crocin ameliorated COX-2, TNF-α (as well as its receptor p-TNF-R1) and iNOS levels and diminished NF-κB-p65 expression, and reduced its level in nuclear extracts; authors indicated NF-κB as a potential regulatory hub inhibited by crocin (Amin, A Hamza et al. 2016). Moreover, following treatment of colon cancer HCT116 cells with

crocin, mRNA expression and secretion of MIP2 (macrophage inflammatory protein 2), IL□8, MCP□1 (monocyte chemoattractant protein-1), TNF□α, IL□6 and IL□1β were reduced. Noteworthy, crocin suppressed protein expression of P□STAT3 which is a regulator of chemokines and inflammatory factors (Kim, An et al. 2017, Wang, Ke et al. 2020).

Main features of the detected preclinical studies testing the potential anti-inflammatory activities of crocin in the gastrointestinal tract, are summarized in Table 3 (Kawabata, Tung et al. 2012, Jnaneshwari, Hemshekhar et al. 2013, El-Maraghy, Rizk et al. 2015, Mard, Nikraftar et al. 2015, HAZMAN, Aksoy et al. 2016, Mard, Pipelzadeh et al. 2016, Hassani, Mehri et al. 2017, Algandaby 2018, Amerizadeh, Rezaei et al. 2018, Faramarzpour, Tehrani et al. 2019, Godugu, Pasari et al. 2019, Hobbenaghi, Ghafarzadeh et al. 2019, Kalantar, Kalantari et al. 2019, Khodir, Said et al. 2019, Luo, Fang et al. 2019, Rezaei, Avan et al. 2019, Samaha, Said et al. 2019, Tawfik and Elkady 2019).

Anti-inflammatory effects in the cardiovascular system

Inflammation and heart failure are markedly associated. In this context, it was shown that increased serum levels of pro-inflammatory cytokines are related with adverse clinical symptoms (Van Linthout and Tschöpe 2017). Thus, suppression of the inflammatory process at an early stage, could prevent chronic inflammation and heart failure progression. Crocin ameliorated myocardial fibrosis features in mice possibly via shutdown of TGF-β1 and TLR4/NF-κB pathways (Jin, Zhang et al. 2020). Previously, contribution of TLR4 and NF-κB to myocardial hypertrophy was shown and it was reported that suppression of NF-κB reduced cardiac fatty acid oxidation and hypertrophy (Yu, Zhang et al. 2015). Besides, TGF-β1 induces cardiac fibroblasts proliferation and transformation (Pan, Zhao et al. 2011). Crocin's cardioprotective effects were also documented by amelioration of histopathological features and reduction of

TNF-α and IL-1β levels with a parallel induction of IL-10 levels in cardiac samples (Elsherbiny, Salama et al. 2016). Pretreatment of embryonic cardiomyocytes (H9c2 cell line) with crocin before LPS exposure, reduced the pro-inflammatory mediators TNF-α, prostaglandin E2 (PGE2), IL-β, and IL-6 levels and decreased TNF-α, COX-2, IL-β, IL-6, and iNOS mRNA levels (Rahim, Khammar et al. 2019).

It was also shown that crocin induces M2 macrophages (which unlike M1 with pro-inflammatory properties, have anti-inflammatory activity) polarization thus, improved coronary atherosclerosis (Li, Lei et al. 2018). In rats with coronary atherosclerosis, crocin decreased IL-6, iNOS and TNF-α but increased IL-4, IL-10 and TGF-β levels in rats' serum. Nuclear levels of NF-κB p65 were decreased in crocin-treated group indicating inhibition of NF-κB p65 translocation (Li, Lei et al. 2018). Besides, in rats with isoproterenol-induced cardiotoxicity, crocin pre-treatment was able to improve edema and inflammatory cells infiltration (Goyal, Arora et al. 2010).

Moreover, crocin protective activity against high glucose-induced injury in human umbilical vein endothelial cells (HUVECs) was mediated by reduction of inflammatory mediator production and suppression of VEGF-A expression (Zhang, Wu et al. 2018).

Moreover, in a rat model of metabolic syndrome, crocin attenuated cardiac and vascular alterations linked with metabolic syndrome and authors attributed the observed effects, at least in part, to crocin anti-inflammatory properties (El-Fawal, El Fayoumi et al. 2019). In STZ-diabetic rats also crocin reduced mRNA levels of TNF-α and IL-6 in abdominal aorta samples (Samarghandian, Azimi-Nezhad et al. 2016).

Crocin effects against inflammation caused by ischemia-reperfusion (IR) were also studied. A complex association between IR injury and allo-immune independent and -dependent immune

responses caused by a complex interaction of innate and adaptive immunity, was shown (Slegtenhorst, Dor et al. 2014).

Crocin's anti-inflammatory properties were evidenced by the reduction of iNOS and COX-2 mRNA levels and IL-1β and TNF-α concentrations (Huang and Jia 2019). Also, crocin in rats with IR-induced (caused by clamping of the celiac artery) gastric mucosal lesions, reduced the protein expression of the pro-inflammatory factor, iNOS (Mard, Nikraftar et al. 2015).

Main features of the detected preclinical studies evaluating the potential anti-inflammatory activities of crocin in the cardiovascular system, are summarized in Table 4 (Elsherbiny, Salama et al. 2016, Samarghandian, Azimi-Nezhad et al. 2016, Li, Lei et al. 2018, Huang and Jia 2019, Rahim, Khammar et al. 2019, Jin, Zhang et al. 2020).

Anti-inflammatory effects in the urogenital system

Inflammation has a critical role in diabetic kidney pathogenesis as evidenced by the increment of renal expression of cell adhesion molecules, growth factors, chemokines and pro-inflammatory cytokines in diabetic individuals (Duran-Salgado and Rubio-Guerra 2014).

In diabetic rats, crocin could down-regulate mRNA and protein expression of NADPH oxidase NOX-4, IL-18 (as a marked pro-inflammatory cytokine), and p53 (which is involved in apoptotic processes), suggesting the potential protective activity of the compound against diabetic nephropathy (Yaribeygi, Mohammadi et al. 2018). Also, crocin decreased TLR-4 and IL-6 in the kidney and reduced NF-κB/p65 positive and TGF-β1 positive cells in a model of diabetic nephropathy in rats (Abou-Hany, Atef et al. 2018).

In kidney samples from STZ-diabetic rats, crocin could ameliorate congestion, inflammation, tubular desquamation, and tubular necrosis as indicated by histological assessments (Altinoz, Oner et al. 2015). Crocin also ameliorated proximal tubules epithelial

539 edema, perivascular edema, and vascular congestion in a rat model of gentamicin-induced
540 nephrotoxicity (Yarijani, Najafi et al. 2016). In an *in vitro* model of diabetic nephropathy, crocin
541 protected D-glucose-treated podocytes by inhibition of NF-κB and reducing the levels of IL-1β,
542 IL-8, IL-10, and TNF-α (Li, Liu et al. 2017).

543 In another experiment, rats with nephrotoxicity induced by tartrazine (a powder dye
544 applied in wool, silk, nylon, leather and paper dyes, toothpaste and hair care products), were
545 treated with crocin; histological assessments indicated that crocin attenuated tubular damage,
546 interstitial inflammation and vascular congestion and significantly lowered total damage score
547 (Erdemli, Gul et al. 2017). In a CCl_4-induced model of nephrotoxicity, crocin lowered IL-6,
548 TNF-α and PGE2 levels in kidney homogenates (Hassan, Bahashawan et al. 2015). PGE2 is
549 known to stimulate secretion of IL-6 and TNF-α (Akira and Kishimoto 1996, Sauter, Wood et al.
550 2011).

551 Also, crocin protected animals from hemorrhagic shock by inhibition of NF-κB (as
552 reflected by augmented nuclear translocation of p65 and IkBa phosphorylation) in the lung,
553 reduced TNF-α and IL-6 serum levels while amplified IL-10 levels, and ameliorated renal
554 dysfunction as evidenced by ameliorated serum creatinine and creatinine clearance (Yang and
555 Dong 2017). Another study that used rats of different ages (i.e. 2, 10 and 20 months old) showed
556 that crocin could reduce TNF-α, IL-6 and IL-1β mRNA and protein expression in aged rats
557 indicating its potential as a protective agent against renal failure (Samarghandian, Azimi□
558 Nezhad et al. 2016).

559 In a study on IR in the kidney, crocin markedly reduced the inflammatory cytokine IL-6
560 and TLR4 renal levels (Abou-Hany, Atef et al. 2018). In another study, rats with renal IR
561 pretreated with crocin, showed suppressed leukocyte infiltration and intercellular adhesion

molecule-1 (ICAM-1) and TNF-α mRNA levels (Yarijani, Pourmotabbed et al. 2017). In another experiment on IR-induced kidney damage, crocin significantly ameliorated TNF-α, IL-6, IL-18, IFN-γ, IL-1β levels in rats kidney (Adali, Gonul et al. 2016). IL-18 is regarded as a trigger to the secretion of IL-1β and IL-6 as well as macrophage inflammatory proteins (MIP-1 / MIP-2) and monocyte chemotactic protein (MCP-1), and an inducer of IFN-γ production (Morel, Park et al. 2002, Dinarello 2004).

In a mice model of endometriosis which has a pathology associated with pro-inflammatory cytokines (Malutan, Drugan et al. 2015), crocin diminished serum INF-γ, TNF-α, and IL-6 concentrations and suppressed vascular endothelial growth factor (VEGF) in both endometriotic lesions and serum samples. Authors showed that crocin reduced secretion of IFN-γ, TNF-α, IL-6 and IL-10 by LPS-exposed human monocytes THP-1 cells (Liu, Qin et al. 2018).

Main features of the detected preclinical studies on the potential anti-inflammatory properties of crocin in the urogenital system, are given in Table 5 (Hassan, Bahashawan et al. 2015, Adali, Gonul et al. 2016, Samarghandian, Azimi□Nezhad et al. 2016, Erdemli, Gul et al. 2017, Yang and Dong 2017, Yarijani, Pourmotabbed et al. 2017, Abou-Hany, Atef et al. 2018, Abou-Hany, Atef et al. 2018, El-Fawal, El Fayoumi et al. 2018, Erdemli, Gul et al. 2018, Liu, Qin et al. 2018, Yaribeygi, Mohammadi et al. 2018).

Anti-inflammatory effects in the musculoskeletal system

Several studies showed abnormal levels of biomarkers such as circulating cytokines, chemokines, and variably C-reactive protein, indicative of systemic inflammation, in preclinical rheumatoid arthritis (RA)(Demoruelle, Deane et al. 2014). Antiarthritic properties of crocin were mediated thorough suppression of p-IκBα, p-IκB kinase (IKK) α/β, and p65 expression and TNF-α, IL-1β, and IL-6 secretion in LPS-treated human fibroblast-like synoviocytes along with

585 diminished serum levels of the afore-mentioned cytokines in mice with arthritis induced by
586 collagen; crocin interaction with IKK led to cessation of NF-κB pathway (Li, Zhang et al. 2018).
587 Also, in rats with type II collagen-induced arthritis, crocin alleviated arthritis scores and paw
588 swelling; based on H&E staining results, with increasing dose of crocin, inflammatory cells
589 number in the joint cavity as well as synovial tissue hyperplasia reduced. Crocin also reduced
590 TNF-α, IL-17, IL-6 and CXCL8 expression in serum and ankle tissues, and diminished MMP-1,
591 -3 and -13 in serum (Liu, Sun et al. 2018). In another study, chondrocytes isolated from rabbit
592 articular cartilage were co-treated with crocin and IL-1β; based on the results, crocin not only
593 suppressed the increment of MMP-1, -3 and -13 expression induced by IL-1β, but also inhibited
594 NF-κB activation by repression of NF-κB-α degradation (Ding, Zhong et al. 2013).

595 It was reported that in rats with osteoarthritis, crocin reduced osteoarthritis-associated
596 joint pain and muscular IL-6 levels while it diminished Nrf2 expression and JNK activity
597 (leading to suppression of NF-κB activation) in mouse skeletal muscle cell line C2C12 (Lei, Guo
598 et al. 2017). The NF-κB and MAPK, with p38, ERK, and JNK are major contributors to principal
599 downstream inflammatory networks (Berenbaum 2004, Avruch 2007).

600 Another study that assessed crocin effects on RA in rats showed reduced paw swelling,
601 arthritis score, iNOS production and serum TNF-α, IL-1β, and IL-6 levels following crocin
602 treatment (Yarijani, Pourmotabbed et al. 2017). Moreover, Freund's complete adjuvant (FCA)-
603 administered rats that received crocins showed attenuated TNF-α and TGF-β1 concentrations in
604 the ankle joint (Hu, Liu et al. 2019). Crocin, in FCA-treated Wistar rats, attenuated elevated
605 concentrations of enzymatic (MMP-13, -3 and -9 and hyaluronidases (HAases)) and non-
606 enzymatic (IL-6, IL-1β, NF-κB, TNF- α, COX-2, PGE2 and ROS) inflammatory factors in serum
607 (Hemshekhar, Santhosh et al. 2012).

Crocin treatment of macrophages derived from mouse bone marrow resulted in suppression of RANKL-induced NF-κB and NFATc1 activation; this effect may be in part due to crocin anti-oxidant properties (Fu, Pan et al. 2017). It was also indicated that 12-week treatment of rats with metabolic syndrome □induced osteoporosis, reduced serum IL□6 and TNF□α concentrations (Algandaby 2019).

Main characteristics of the detected preclinical studies reporting potential anti-inflammatory effects of crocin in the musculoskeletal system, are summarized in Table 6 (Fu, Pan et al. 2017, Lei, Guo et al. 2017, Yarijani, Pourmotabbed et al. 2017, Li, Zhang et al. 2018, Algandaby 2019, Hu, Liu et al. 2019, Zhu, Yang et al. 2019, Wang, Xu et al. 2020).

Miscellaneous

In an *ex vivo* study, crocin effect on venous blood from healthy subjects treated with viper venom was assessed; crocin reduced serum TNF-α, IL-1β, IL-6 and COX-2 levels (Santhosh, Sundaram et al. 2013). Nonetheless, treatment of LPS-treated RAW 264.7 with crocin, produced no significant reduction in COX-2-mediated PGE2 and iNOS-mediated NO production (Lim, Park et al. 2008). Moreover, crocin could ameliorate histopathological changes in rats with experimental periodontitis (Kocaman, Altinoz et al. 2019).

Another *in vitro* study reported reduced IL-8, PGE2, IL6, TNF-α, IL-1α and LTB4 secretion by NHEKs (both preventive and curative while for IL1α it was only preventive) and decreased IL-1 signaling pathway, following treatment of human keratinocytes NHEKs and human dermal fibroblasts with crocin (Fagot, Pham et al. 2018). In this study, a moderate induction of *NFKBIA* expression, which codes for IKB in NHEKs keratinocytes was shown; this evidence implies that by using crocin, NK-κB is retained in the cytoplasm and its activation is suppressed. Also, crocin suppressed *IL1-A* expression which encodes for IL1-α which is a major

631 contributor to inflammation. Stoppage of IL1 pathway was further documented by lower gene
632 expression of TNF-α, CC α-chemokines (CXCL1 and CXCL2), STAT1, PDGFB (platelet-
633 derived growth factor) and PLAU (urokinase-type plasminogen activator), all playing a role in
634 inflammation and fibrosis production. Moreover, crocin down-regulated the expression of MMP-
635 9 encoding gene (Fagot, Pham et al. 2018).

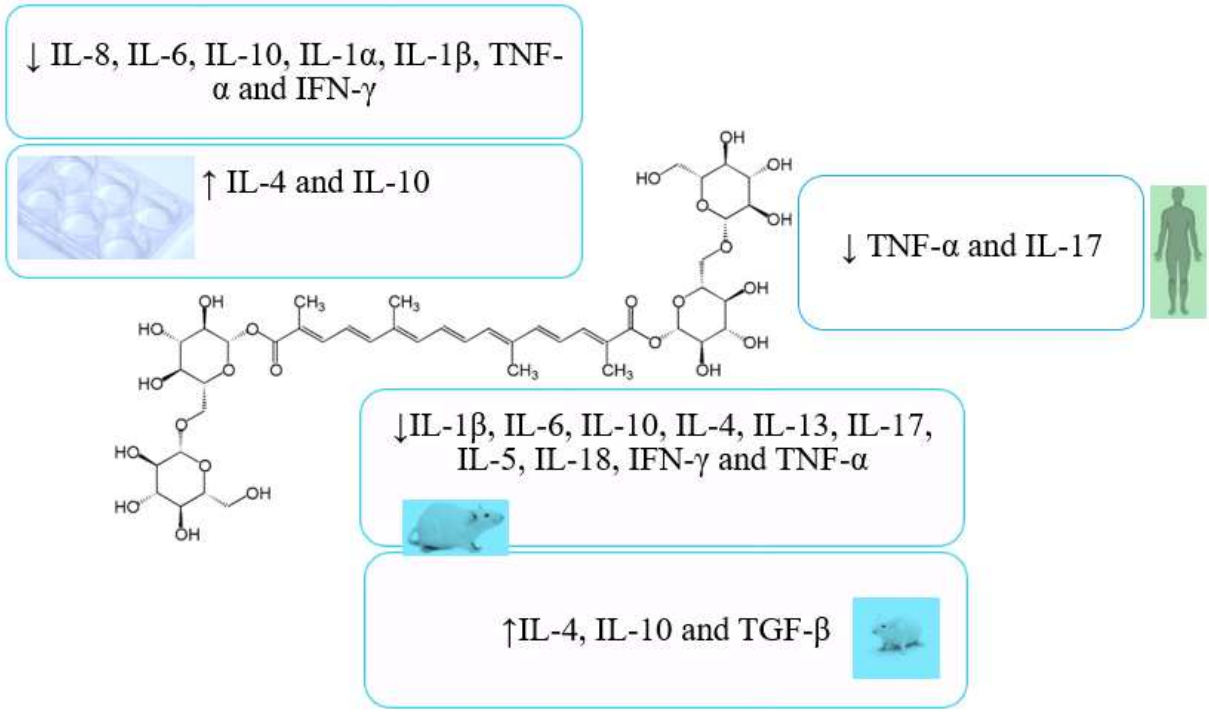
636 Sung and Kim reported alleviative effects of crocin against with *Dermatophagoides*
637 *farinae* crude extract-induced atopic dermatitis in NC/Nga mice; their results indicated that
638 crocin suppressed NF-κB/STAT6 signaling and reduced dermatitis severity, and serum
639 concentrations of IgE, and Th2 cytokines (IL-4 and IL-5), but not Th1 cytokines (IFN-γ and IL-
640 12) (Sung and Kim 2018).

641 Pro-inflammatory factors like IL-1, LPS, and TNF induce NF-κB formation and
642 translocation; crocin, in a rat model of aging, suppressed hippocampal levels of IL-1, TNF-α and
643 NF-κB (Heidari, Mehri et al. 2017). Similarly, crocin suppressed serum levels of IL-6 and TNF-
644 α in mice in a study on D-galactose-induced aging (Mohammadi, Mehri et al. 2018).

645 Xu et al. conducted *in vitro* (RAW 264.7 cells) and *in vivo* (male Kunming mice) studies
646 on anti-inflammatory properties of crocin and found that crocin reduced COX-1 and COX-2 and
647 PGE2 in LPS-treated cells and decreased nuclear translocation of the NF-κB p50 and p65, and *in*
648 *vivo*, it ameliorated ear edema (caused by xylene treatment) and paw edema (caused by
649 carrageenan treatment) as well as gastric lesions (Xu, Li et al. 2009).

650 **Figures 2** and **3**, respectively depict various cytokines and multiple pathways involved in crocin
651 anti-inflammatory activities.

652



653

654

655 **Figure 2.** Modulatory effects of crocin on the various cytokines involved in

656 inflammation.

657

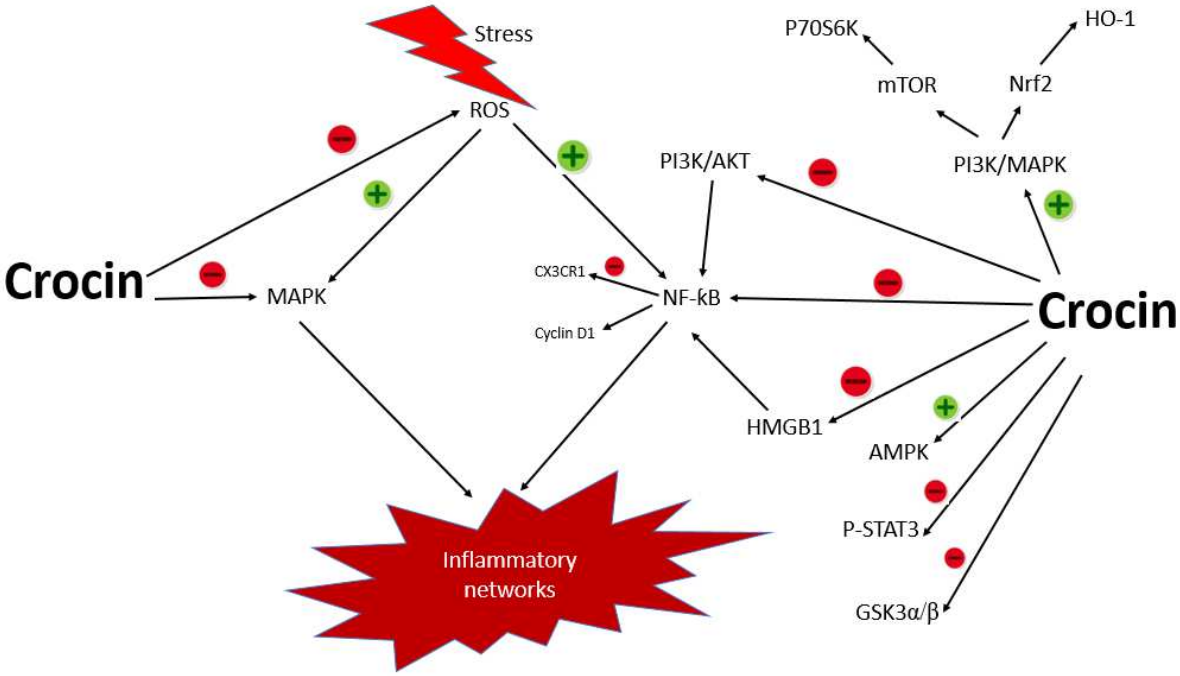


Figure 3. Crocin anti-inflammatory activities are mediated via multiple pathways.

Anti-nociceptive effects of crocin

Since crocin has shown anti-nociceptive effects in various models, here, we discuss such effects reported by the retrieved studies. Generally, pain syndrome possesses an inflammatory profile that comprises a series of inflammatory factors; in other words "*The origin of all pain is inflammation and the inflammatory response*" (Omoigui 2007). In this context, ample evidence from animal models confirmed an analgesic activity for anti-inflammatory cytokines (Sommer, Leinders et al. 2018). Although the etiologies of inflammatory pain and neuropathic pain are essentially dissimilar, doubtlessly, these conditions share mutual pathways that produce pain state (Xu and Yaksh 2011). Besides, the marked role of nitro-oxidative stress in development of neuropathic pain was shown (Carrasco, Naziroğlu et al. 2018) and effects of natural compounds against pain state, were reported (Hashemzaei, SadeghiBonjar et al. 2015, Hassani, Rezaee et al. 2015, Hashemzaei, Abdollahzadeh et al. 2017, Rezaee, Monemi et al. 2019).

Safakhah et al. employed a neuropathic pain model induced by chronic constriction injury (CCI) and found that 14-day administration of crocin decreased thermal hyperalgesia and mechanical allodynia; authors suggested that antioxidant/anti-inflammatory properties of crocin contributed to the observed anti-nociceptive effects (Safakhah, Taghavi et al. 2016). Also, crocin was given to rats used as a contusion model of spinal cord damage. Crocin improved results of mechanical behavior and locomotor recovery tests but did not affect those of thermal behavioral test. Also, serum levels of CGRP (calcitonin gene related peptide) were lowered by crocin. CGRP which is recognized as the most effective vasodilator, plays a role in inflammatory processes and chronic pain (Karami, Bathaie et al. 2013). In another study, crocin alleviated hyperalgesia through spinal Wnt5α/β-catenin signaling (Wang, Xu et al. 2020). This pathway can influence RA by modulating fibroblast-like synoviocytes proliferation, inflammatory factors

secretion and metabolism/ destruction of bones (de Sousa, dos Santos Alves et al. 2019, Liang, Li et al. 2019). Wnt protein family is involved in development of neuropathic pain. Under acute and chronic pain condition, Wnt5a is involved in central sensitization and neuronal plasticity (Li, Fan et al. 2018, Rong, Liu et al. 2019). Activation of Wnt5a/β-catenin stimulates expression of TNF-α, IL-1β and COX-2 (which regulates the expression of iNOS) (Yang, Chu et al. 2014, Shi, Man et al. 2016, Wu, Zhang et al. 2017, Yuan, Shi et al. 2018).

Crocin also had beneficial effects against STZ-induced cold allodynia and edema in rats (Farshid and Tamaddonfard 2015). Also, in rats, intra-fourth ventricle injection of crocin in a model of capsaicin-induced orofacial pain as well as intraperitoneal injection of crocin in a model of formalin-induced orofacial pain, exhibited anti-nociceptive properties (Erfanparast, Tamaddonfard et al. 2015). Treatment of rats with local inflammation (caused by intraplantar carrageenan) with crocin, led to amelioration of edema and inflammatory pain responses and lower neutrophils numbers (Tamaddonfard, Farshid et al. 2013). Moreover, in rats with hypertonic saline-induced corneal pain, intraperitoneal and intracerebroventricular (icv) crocin produced anti-nociception which was concluded not to be mediated through opioid receptors (Tamaddonfard and Hamzeh□Gooshchi 2010). Crocin also attenuated pain in rats that received intra-plantar injection of formalin, and enhanced morphine-induced anti-nociception (Tamaddonfard and Hamzeh□Gooshchi 2010). In a recent study, crocin was shown to preserve morphine analgesic potential and avoid development of tolerance towards morphine in neuropathic pain; authors suggested that such effects are independent of BDNF activity (Safakhah, Damghanian et al. 2020). However, a report showed that crocin (even at 50 mg/kg) could not relieve CCI-induced neuropathic pain in rats (Amin and Hosseinzadeh 2012).

Intervertebral disc (IVD) degeneration is known to be associated with low back pain (LBP) (Molinos, Almeida et al. 2015). Since inflammation is a major player in the occurrence of IVD degeneration (Molinos, Almeida et al. 2015), intradiscal crocin was administered to rats with IVD degeneration; Crocin was found to have beneficial effects against IVD degeneration as reflected by the suppression of type II collagen, aggrecan, MMP-3 and ADAMTS-5 mRNA levels (Li, Li et al. 2016).

In an *in vitro* study, nucleus pulposus (NP) cells from rats lumbar IVDs were treated with crocin and LPS. Crocin attenuated LPS-induced increases in catabolic enzymes (MMP-1, -3, MMP-13, as well as a disintegrin-like and metalloprotease (reprolysin type) with thrombospondin type 1 motif (ADAMTS)-4 and ADAMTS□5), and IL-1β, TNF-α, IL-6, iNOS and TLR□2. Also, by suppressing the phosphorylation of JNK, crocin inhibited MAPK pathway. Crocin, to some extent, prevented decrement of aggrecan and type II collagen. These findings propose crocin as a possible treatment for LBP (Molinos, Almeida et al. 2015). Figure 4 presents pathway that were shown to be involved in anti-nociceptive functions of crocin.

The pathways involved in crocin anti-nociceptive activities, are depicted in **Figure 4**.

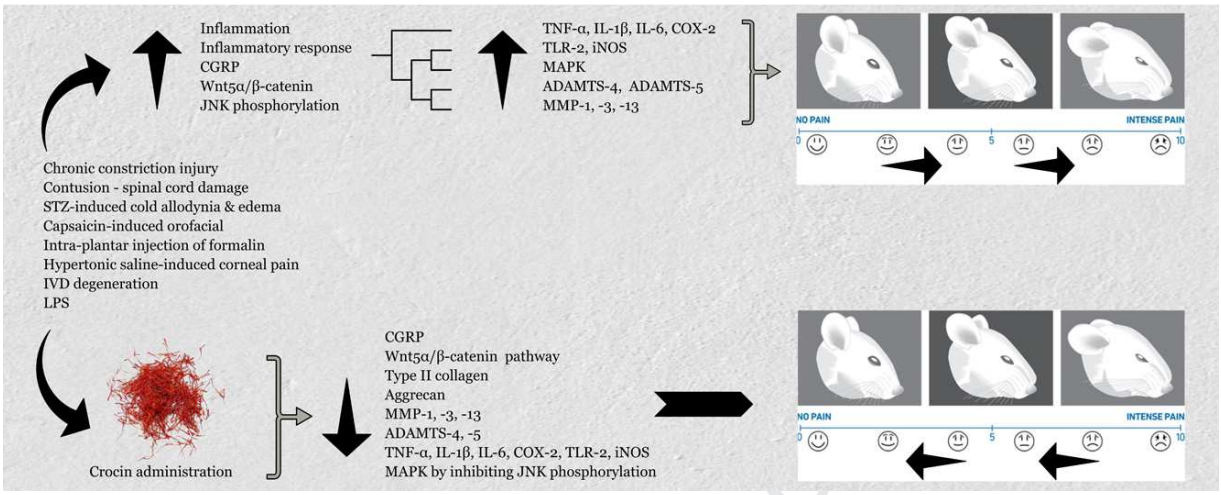


Figure 4. Crocin induces its anti-nociceptive effects by modulation of multiple pathways.

Conclusions

Crocin not only combats ROS production and suppresses pro-inflammatory cytokines secretion but also alleviates inflammation in various organs/systems, as shown by a series of animal models and *in vitro* experiments, via regulating mainly NF-κB pathway and NF-κBp65 translocation to cell nucleus. In this context, modulation of PI3K/Akt appears to be a favorable crocin target contributing to NF-κB pathway inhibition. The anti-inflammatory/anti-nociceptive effects of crocin discussed in the present article warrant further testing in clinical setting.

732 **Table 1.** Preclinical studies reporting potential anti-inflammatory effects of crocin in the
733 respiratory tract.

Species/model	**Crocin dose/route of administration/duration of treatment**	**Main effects/outcomes (and underlying mechanisms)**	**Reference**
C57BL/6 mice with cigarette smoke-induced COPD	50 mg/kg/ ip/ once daily	↓ numbers of inflammatory cells, suppressed peribronchial inflammatory cells infiltration, ↓ pro-inflammatory cytokines levels in BALF ↓ levels of IL-1β, IL-6 and TNF-α in the lung tissue	(Xie, He et al. 2019)
Sprague-Dawley rats with cigarette smoke-induced COPD	50 mg/kg/day ip, three times/week for two months	↓ number of macrophages and lymphocytes in BALF; restored BALF IL-6 and TNF-α levels; ↑ PKC, PI3K, MAPK and Nrf2 mRNA expression	(Dianat, Radan et al. 2018)
Swiss Albino mice with OVA-induced asthma	25 mg/kg, oral, for 16 days	↓ number of monocytes, neutrophils, and eosinophils; diminished lung TNF-α, IL-4 and IL-13 levels; ameliorated perivascular edema, peribronchial inflammation, and macrophage infiltration in the alveolar space	(Yosri, Elkashef et al. 2017)
BALB/c mice with OVA-induced allergic airway inflammation	100 mg/kg, intragastrically	↓ total and differential leukocyte counts; Inhibited inflammatory cell infiltration and mast cell degranulation; ↓ IL-4, IL-5, IL-13, OVA-specific IgE and ↓expression of p38 MAPK, JNK and ERK in the lung.	(Xiong, Wang et al. 2015)
C57BL/6 mice with LPS-induced ARDS LPS-stimulated HUVECs	15, 30, and 60 mg/kg, ip, pretreatment for 7 days pretreated with 20 μmol/l	↓ neutrophil infiltration and lung injury; ↓ MMP□9 expression *in vivo* but not *in vitro*; inhibited the phosphorylation of p38, ERK, and JNK in lungs; IκBα phosphorylation, NF-κB p65 nuclear transfer, and HMGB1 cytoplasmic transfer	(Zhang, Qi et al. 2020)
Mice with LPS-induced acute lung	50 mg/kg intragastric injection 1 hr before LPS	↓ IL-1β and TNF-α in BALF; ↓ LPS-induced activation of iNOS in the	(Xiong, Wang et al. 2015)

injury		lung;	
BEAS-2B and NHBE cells	1 μM for 24 hr	↓TNF-α, IL-8, IL-6 and IL-1β; Inhibition of NF-κB p65 nuclear translocation; IKKα and IκBα phosphorylation	(Du, Chi et al. 2018)
Murine macrophage RAW 264.7 Cells treated with LPS	100, 250 and 500 μM	↑HO-1 expression; ↓ iNOS expression and NF-κB activation; Activated Nrf2	(Kim, Park et al. 2014)
Rats treated with bleomycin (5 mg/kg) infused into the Trachea	20 mg/kg/day, orally, one week prior to and 4 weeks post bleomycin exposure	↓ TLR4 and IL-10 expression	(Zaghloul, Said et al. 2019)

Abbreviations: IL: Interleukin; TLR4: Toll-Like Receptor 4; TNF: Tumor necrosis factor; iNOS: Inducible nitric oxide synthase; HO-1: Heme oxygenase-1; ARDS : Acute respiratory distress syndrome ; LPS : Lipopolysaccharide ; NF-κB : Nuclear Factor-kappa B ; MMP: Matrix metalloprotease ; HMGB1: High mobility group box protein 1 ; IgE: Immunoglobulin E ; ip: intraperitoneal ; OVA: ovalbumin ; MAPK: Mitogen-activated protein kinase ; JNK: c-Jun N-terminal Kinase ; ERK: extracellular signal-regulated kinases; PKC: Protein kinase C ; Nrf2: nuclear factor erythroid derived 2 like 2 ; COPD: Chronic obstructive pulmonary disease; PI3K: Phosphoinositide 3-kinase; HUVECs: human umbilical vein endothelial cells; BEAS-2B: Human bronchial epithelial cell line; NHBE: normal human bronchial epithelial cells

742 **Table 2.** Preclinical studies investigating the potential anti-inflammatory properties of crocin in
743 the nervous system.

Species/model	Crocin dose/route of administration/duration of treatment	Main effects/outcomes (and underlying mechanisms)	Reference
C57BL/6 mice with cigarette smoke-induced COPD	50 mg/kg, ip, once a day	↓ levels of smoke-induced IκB phosphorylation and degradation, inhibition of NF-κBp65 translocation to the nucleus; restored augmented hippocampal concentrations of pro-inflammatory cytokines	(Xie, He et al. 2019)
Parkinsonian rats induced by 6-OHDA	100 mg/kg, ip, started 1 week before 6-OHDA administration and continued until 6 weeks after it.	↓ TNF-α in the striatum ↓ lipid peroxidation in the hippocampus (at 100 mg/kg)	(Shahidani, Rajaei et al. 2019)
Wistar rats with rotenone-induced Parkinsonism	30 mg/kg/day, ip, for 30 days	↑ striatal phospho and total PI3K, Akt, PRAS40, p70S6K, mTOR, GSK3β, and FoxO3a; ↑ mTOR, miRNA-7, and miRNA-221 ↓ GSK3β, and FoxO3 gene expression.	(Salama, Abdel-Latif et al. 2020)
Rats with methamphetamine-induced neurodegeneration	10, 20, 40 and 80 mg/kg ip, for 21 days	At 40 and 80 mg/kg, ↓ TNF-α and IL-1β in hippocampal lysates.	(Mozaffari, Yasuj et al. 2019)
C57BL/6J mice with chronic corticosterone -induced depression	20 and 40 mg/kg orally for 2 weeks *crocin-I	↓ hippocampal expression of IL-6 and IL-1β mRNA and IL-1β protein;	(Xiao, Xiong et al. 2019)
Wistar rats with Alzheimer's disease induced by trimethyltin chloride	0.5 ml at 25 and 50 mg/kg (?) for 30 days	↓ IL-6, IL-1β, and TNF-α in serum ↑neuronal density of hippocampal CA1, CA2 and CA3.	(Sadoughi 2019)
Male C57BL/6 mice with TBI	20 mg/kg 30 min before TBI	↓ number of activated microglia and levels of IL-1β and TNF-α; Activated Notch signaling	(Wang, Zhang et al. 2015)
Female Wistar rats	150 mg/kg ip 2 weeks	↓ serum CGRP	(Karami, Bathaie

with contusive spinal cord injury	after injury		et al. 2013)
LPS-treated BV-2 microglia	200 μM for 24 hr	↓ expression of IL-6, CCL2 and iNOS; No significant impact on NF-κB nuclear translocation.	(Yorgun, Rashid et al. 2017)
BV-2 and N9 cells treated with high glucose and free fatty acid	0.1 or 1 μM for 24 hr	↓ iNOS and COX-2 mRNA and protein levels; ↑ PI3K and p-Akt proteins; ↓ IL-1β and TNF-α	(Yang, Huo et al. 2017)
LPS-treated Kunming mice	10, 20 and 40 mg/kg given ip for 7 days prior to LPS administration	↓ IL-1β, IL-18, and TNF-α concentrations and NLRP3 activation	(Zhang, Previn et al. 2018)
Wistar rats treated with methylphenidate	10, 20, 40, and 80 ip mg/kg for 21 days	↓ hippocampal IL□1β and TNF□α, ↑ induced P□CREB, and BDNF in the hippocampus.	(Ebrahimzadeh, Moghadam et al. 2019)
Rat model of LPS-induced septic shock	10 mg/kg and 100 mg/kg) given 30 min before LPS	↓ liver and brain concentrations of PAI-1	(Tsantarliotou, Lavrentiadou et al. 2019)
Methamphetamine-treated Wistar rats	Four doses of 30, 60 or 90 mg/kg ip with 2-hr intervals.	↓ MDA and TNF-α concentrations; suppressed caspase□3 activation in hippocampus samples.	(Shafahi, Vaezi et al. 2018)
Malathion-exposed Wistar rats	10, 20, and 40 mg/kg/day ip for 4 weeks	↓ striatum levels of TNF-α and IL-6	(Mohammadzadeh, Hosseinzadeh et al. 2018)
Wistar rats with D-galactose-induced aging	7.5, 15, 30 mg/kg ip for 8 weeks	↑ pAkt/c and pERK/ERK ratio; ↓ hippocampal NF-κB p65, TNF-α and IL-1β	(Heidari, Mehri et al. 2017)

Abbreviations: IL: Interleukin; TNF: Tumor necrosis factor; iNOS: Inducible nitric oxide synthase; LPS: Lipopolysaccharide; NF-κB: Nuclear Factor-kappa B; ip: intraperitoneal; COPD: Chronic obstructive pulmonary disease; PI3K: Phosphoinositide 3-kinase; MDA: Malondialdehyde; CREB: cAMP response element binding protein; BDNF: Brain derived neurotrophic factor; PAI-1: plasminogen activator inhibitor type 1; NLRP3: NLR family pyrin domain-containing protein 3; COX: cyclooxygenase; CCL2: CC-chemokine ligand 2; CGRP: calcitonin gene related peptide; TBI: traumatic brain injury; mTOR: mammalian target of rapamycin; GSK3β: Glycogen synthase kinase-3 beta; FOXO3: forkhead box O3; PRAS40: proline-rich Akt substrate of 40 kDa; 6-OHDA: 6-hydroxydopamine.

753 **Table 3.** Preclinical studies investigating the potential anti-inflammatory effects of crocin in the
754 gastrointestinal system.

Species/model	Crocin dose/route of administration/duration of treatment	Main effects/outcomes (and underlying mechanisms)	Reference
Methotrexate-treated rats	25 and 50 mg/kg, oral, for 9 days	↓ hepatic levels of IL-1β and TNF-α	(Kalantar, Kalantari et al. 2019)
Rats with AA-induced UC	20 mg/kg, oral, for 8 days finishing on the day UC was induced	↓ CRP level in colon homogenate.	(Khodir, Said et al. 2019)
Wistar rats with AA-induced UC	5, 10 and 20 mg/kg, ip, for 8 days And 5 mg/kg crocin+100 mg/kg mesalazine	Alone and in combination with mesalazine ↓ edema, inflammatory cell infiltration and crypt destruction; ↓ colonic concentrations of TNF-α.	(Faramarzpour, Tehrani et al. 2019)
C57BL/6 male mice with DSS-induced colitis	50 and 200 ppm in drinking water, for 3 weeks	Improved colitis histological features and suppression of inflammation. ↓ serum and colon levels of high-sensitivity CRP (hs-CRP). ↓ DSS-induced collagen deposition (i.e. fibrosis). Inhibition of DSS-induced increment of cyclin D1 expression and GSK3α/β phosphorylation.	(Rezaei, Avan et al. 2019)
C57BL/6 female mice with DSS-induced colitis□associated colon cancer	200 ppm in drinking water	↓ MDA levels, improved histopathological features and ↓expression of PI3K and cyclin D1 and surviving. suppressed Akt and GSK3α/β phosphorylation.	(Amerizadeh, Rezaei et al. 2018)
ICR mice with DSS-induced colitis	0, 50, 100, and 200 ppm orally for 4 weeks	↓ inflammation scores in the large bowel; ↓ COX-2, iNOS, IFN-γ, TNF-α, IL-1β, IL-6 and NF-κB mRNA expression, ↑ Nrf2 mRNA levels.	(Kawabata, Tung et al. 2012)
Swiss albino mice with cerulein-induced AP	30 and 100 mg/kg, ip, for 7 days prior to cerulein administration	↓ AP-induced increases in pancreas weight, plasma amylase levels, neutrophil infiltration and pancreatic levels of IL-1β, IL-6, IL-17,	(Godugu, Pasari et al. 2019)

		and TNF-α; suppressed cytosolic and nuclear levels of p65-NFκB; ↑Nrf2; ameliorated pancreatic inflammation, necrosis, and acinar edema.	
Wistar albino rats with BPA-induced liver toxicity	5, 10, or 20 mg/kg, ip, for 30 days	↓ level of miR-122, and protein and phospho-protein expression of JNK, ERK1/2 and P38), MAPKAPK and AKT (but did not affect Akt phosphorylation); ameliorated condensed nuclei, substantial leukocytes infiltration, and periportal inflammation.	(Hassani, Mehri et al. 2017)
Rats with IR-induced gastric lesions	15 mg/kg, ip, 30 min before IR induced by clamping the celiac artery	↓protein expression of active caspase-3 and iNOS; ↓ total area of mucosal lesions;	(Mard, Nikraftar et al. 2015)
Swiss albino rats with hepatic toxicity due to γ-rays (6Gy) exposure	200 mg/kg, ip, for 7 days	↓ ALT and AST as well as ALP, TNF-α, and IL-1β in serum Restoration of normal architecture of hepatic tissues.	(Tawfik and Elkady 2019)
Wistar rats with cyclophosphamide-induced hepatotoxicity	10 mg/kg, oral, for 6 days	↓ IL-1β, IL-6, TNF-α and COX-2 in serum.	(Jnaneshwari, Hemshekhar et al. 2013)
Rats with ethanol-induced gastric injury	50 mg/kg ip, for 3 days	↑ gastric mucosal levels of PGE2 and IL-6; ↓ mRNA and protein expression of HSP70; ↓ TNF-α; ↓ neutrophil infiltration;	(El-Maraghy, Rizk et al. 2015)
Wistar rats with indomethacin-induced small intestinal ulcer	2.5, 10 and 40 mg/kg ip, 1, 4, and 16 hr post indomethacin administration	↓levels of TNF-α and caspase-3 in small intestine samples	(Hobbenaghi, Ghafarzadeh et al. 2019)
Mice with thioacetamide-induced liver fibrosis	25 and 100 mg/kg/day, oral, for 6 weeks	↓ gene expression of TGF-β1 and a-SMA andcollagen-1; ↑ MMP-2 gene expression. ↓ levels of NF-κB and COX-2; ↓ levels of IL-1β and TNF-α	(Algandaby 2018)

Wistar rats with indomethacin-induced gastric erosion	7.5, 15 or 30 mg/kg, ip, 30 min prior to indomethacin	↓ mRNA expression of COX-1, COX-2, and iNOS	(Mard, Pipelzadeh et al. 2016)
C57BL/KsJ-Lepdb (db/db) mice with NAFLD	20 mg/kg orally for 8 weeks	Induction and inhibition of AMPK and mTOR phosphorylation, respectively, ↓ serum levels of alanine aminotransferase; improvement of hepatic injury	(Luo, Fang et al. 2019)
Sprague-Dawley rats with STZ-induced diabetes	10 mg/kg orally for 4 weeks	↓ CD68 expression indicating less macrophages within the β-islets.	(Samaha, Said et al. 2019)
High Fat Diet-administered obese group treated with STZ	150 mg/kg orally for 6 weeks	↓ serum TNF-α and IL-1β ↓ TNF-α and IFN-γ in pancreas tissue.	(HAZMAN, Aksoy et al. 2016)

Abbreviations: IL: Interleukin; TLR4: Toll-Like Receptor 4; TNF: Tumor necrosis factor; iNOS: Inducible nitric oxide synthase; NF-κB: Nuclear Factor-kappa B; MMP: Matrix metalloprotease; ip: intraperitoneal; MAPK: Mitogen-activated protein kinase; JNK: c-Jun N-terminal Kinase; ERK: extracellular signal-regulated kinases; PI3K: Phosphoinositide 3-kinase; MDA: Malondialdehyde; COX: cyclooxygenase; mTOR: mammalian target of rapamycin; DSS: dextran sodium sulfate; AA: Acetic acid; UC: Ulcerative colitis; CRP: C Reactive Protein; AMPK: 5'-adenosine monophosphate (AMP)-activated protein kinase; GSK3α/β: glycogen synthase kinase 3α/β; IFN: Interferon; AP: acute pancreatitis; BPA: Bisphenol A; IR: Ischemia/Reperfusion; ALP: alkaline phosphatase; PGE2: Prostaglandin E2; HSP: Heat shock protein; TGF: Transforming growth factor; STZ: Streptozotocin; NAFLD: Non-alcoholic fatty liver disease.

769 **Table 4.** Preclinical studies reporting the potential anti-inflammatory effects of crocin in the
770 cardiovascular system.

Species/model	Crocin dose/route of administration/duration of treatment	Main effects/outcomes (and underlying mechanisms)	Reference
Mice with myocardial fibrosis caused by isoproterenol	100 and 200 mg/kg/day, ip, for 14 days	↓ IL-1 and -6 and TNF-α as well as NF-κB (p65), TLR4 caspase-3, and cleaved caspase-3 expressions. Improvement of cellular swelling, and focal inflammatory cells infiltration.	(Jin, Zhang et al. 2020)
Sprague-Dawley rats with doxorubicin-induced cardiotoxicity	10 and 20 mg/kg, oral, for 3 weeks	↓ interstitial and perivascular fibrosis and TNF-α and IL-1β levels in myocardial samples; ↑ myocardial IL-10	(Elsherbiny, Salama et al. 2016)
Embryonic cardiomyocytes (H9c2) with LPS-induced toxicity	10, 20 and 40 μM for 24 hr prior to LPS	↓ expression of TNF-α, PGE2, IL-β, and IL-6 at protein and expression of TNF-α, COX-2, IL-β, IL-6, and iNOS at mRNA levels.	(Rahim, Khammar et al. 2019)
C57BL/6J mice that underwent left common carotid artery ligation	5, 10 or 20 mg/kg immediately or 2 hours post hypoxia–ischemia	Non-significant ↓ of iNOS and COX-2 mRNA expression stimulated by hypoxia–ischemia. ↓ brain levels of IL-1β and TNF-α.	(Huang and Jia 2019)
Wistar rats with vitamin D3-induced coronary atherosclerosis	100 mg/kg/day for 4 weeks	↓ serum IL-6, iNOS and TNF-α concentrations; ↑ IL-4, IL-10 and TGF-β levels; ↓ NF-κB p65 expression and translocation; ↑ M2 macrophage polarization	(Li, Lei et al. 2018)
Rats with STZ-induced diabetes	10, 20, 30 mg/kg/day, ip, for 4 weeks	(in abdominal aorta samples) ↓ mRNA expression of TNF-α and IL-6	(Samarghandian, Azimi-Nezhad et al. 2016)

771 **Abbreviations:** IL: Interleukin; TLR4: Toll-Like Receptor 4; TNF: Tumor necrosis factor; iNOS: Inducible nitric
772 oxide synthase; NF-κB: Nuclear Factor-kappa B; COX: cyclooxygenase; TGF: Transforming growth factor; STZ:
773 Streptozotocin.
774

775

776 **Table 5.** Preclinical studies investigating the potential anti-inflammatory effects of crocin in the
777 urogenital system.

Species/model	Crocin dose/route of administration/duration of treatment	Main effects/outcomes (and underlying mechanisms)	Reference
STZ-induced diabetic Wistar rats	40 mg/kg, ip, for 8 weeks	↓ IL-18, NOX-4 and P53 expression (mRNA and protein).	(Yaribeygi, Mohammadi et al. 2018)
Rats with fructose-induced metabolic syndrome	50 mg/kg, oral, for 10 weeks	↓ serum concentrations of TNF-α ↓ foci of mononuclear inflammatory cells in kidney cortex	(El-Fawal, El Fayoumi et al. 2018)
Wistar rats with CCl_4-induced nephrotoxicity	100 mg/kg/day, oral, for 15 days	Significant improvement of histological findings (e.g. inflammatory cells infiltration)	(Erdemli, Gul et al. 2018)
Rats with CCl_4-induced nephrotoxicity	100 mg/kg/day, ip, for 3 weeks	↓ renal IL-6 and TNF-α; Ameliorated vascular congestion; ↓ CCl_4–induced increased levels of PGE2	(Hassan, Bahashawan et al. 2015)
Sprague–Dawley rats with STZ-induced diabetes	20 mg/kg/day, oral, for 8 weeks	↓ renal levels of hydroxyproline and collagen; ↓ renal content of TLR4 and IL-6; ↓ NF-κB/p65 positive and TGFβ1 positive cells.	(Abou-Hany, Atef et al. 2018)
Differentiated podocytes seeded in type 1 collagen-coated plates and supplemented with D-glucose	0.1, 0.5, or 1 μM	↓ IL-1β, IL-8, IL-10, and TNF-α; ↓ expression of p-IκBα	(Erdemli, Gul et al. 2017)
Wistar rats (2, 10 and 20 months old)	10, 20 and 30 mg/kg/day for 28 days	↓ TNF-α, IL-6 and IL-1β mRNA expression in renal tissues; ↓ their serum levels in old rats;	(Samarghandian, Azimi□Nezhad et al. 2016)
Sprague–Dawley rats with URIRI	20 mg/kg, oral, for 7 days prior to URIRI	↓ TLR4 and IL-6 levels as well as MDA and NO contents in kidney samples; ↓ inflammatory cells number.	(Abou-Hany, Atef et al. 2018)
Wistar rats with abdominal aortic ischemia	100 mg/kg, ip, 30 min before laparotomy	↓ renal TNF-α, IL-6, IL-18, IFN-γ, IL-1β; ameliorated cellular vacuolization and interstitial edema in the kidney	(Adali, Gonul et al. 2016)

Wistar rats	A single ip dose of 100, 200, or 400 mg/kg	↓ leukocyte infiltration into the renal interstitium, as well as TNF-α and ICAM-1 mRNA expression	(Yarijani, Pourmotabbed et al. 2017)
Female Balb/c mice with endometriosis	25 mg/kg, ip, for 3 weeks	↓ serum INF-γ, TNF-α, VEGF and IL-6. *In vitro* (20 μM): ↓ secretion of IFN-γ, TNF-α, IL-6 and IL-10 by LPS-exposed human monocytes THP-1 cells.	(Liu, Qin et al. 2018)
Wistar rats underwent hemorrhagic shock	60 mg/kg iv	↓ nuclear translocation of p65; ↑ IkBα phosphorylation in lung tissue; ↓ serum TNF-α and IL-6; ↑ IL-10 levels	(Yang and Dong 2017)

Abbreviations: IL: Interleukin; TLR4: Toll-Like Receptor 4; TNF: Tumor necrosis factor; NF-κB: Nuclear Factor-kappa B; ip: intraperitoneal; MDA: Malondialdehyde; IFN: Interferon; PGE2: Prostaglandin E2; TGF: Transforming growth factor; STZ: Streptozotocin; NOX: NADPH oxidase; CCl4: carbon tetrachloride; URIRI: Unilateral renal ischemia reperfusion injury; ICAM: Intercellular adhesion molecules; VEGF: vascular endothelial growth factor.

788 **Table 6.** Preclinical studies investigating the potential anti-inflammatory effects of crocin in the
789 musculoskeletal system.

Species/model	Crocin dose/route of administration/duration of treatment	Main effects/outcomes (and underlying mechanisms)	Reference
RAW 264.7 cells	0, 40, or 80 μM	↓ p38 and JNK signaling, Ti particle-induced inflammation was suppressed; ↑ osteogenic differentiation of Bone mesenchymal stem cells by inducing M2 macrophage polarization	(Zhu, Yang et al. 2019)
Mice with collagen-induced arthritis LPS-stimulated human fibroblast-like synoviocytes	50 mg/kg/daily, ip, for 1 week (*in vitro*) 0, 10, 100, 200, and 500 μM	↓ serum TNF-α, IL-1β, and IL-6 ↓ TNF-α, IL-1β, and IL-6 ↓ p-IKKα/β, p-IκBα, and p65 proteins expression by the cells	(Li, Zhang et al. 2018)
Wistar rats with type II collagen-induced arthritis	10, 20 or 40 mg/kg continued for 36 days	↓ TNF-α, IL-17, IL-6 and CXCL8 in serum and ankle tissues ↓ serum levels of MMP-1, -3 and -13; ↓arthritis scores and paw swelling	(Li, Zhang et al. 2018)
Sprague-Dawley rats with meniscectomy-induced osteoarthritis Mouse skeletal muscle cell line C2C12	30 mg/kg/day, oral, for 10 days	↓ muscular level of IL-6 slight attenuation of JNK phosphorylation	(Lei, Guo et al. 2017)
Sprague-Dawley rats with Freund's complete adjuvant arthritis	50 and 100 mg/kg ip	↓ spinal TNF-α and IL-1β; ↓ the Wnt5a/β-catenin pathway;	(Wang, Xu et al. 2020)
Rats with rheumatoid arthritis	6.25, 12.5 and 25 mg/kg/ day orally for 28 days	(in arthritis synovium samples): ↓ paw redness and swelling; ↓ arthritic score and iNOS mRNA and protein expression; ↓ serum IL-1β, IL-6, and TNF-α	(Yarijani, Pourmotabbed et al. 2017)
Sprague-Dawley rats	160 mg/kg, oral, for 14	↓ synovial tissue TNF-α and TGF-β1;	(Hu, Liu et al.

with Freund's complete adjuvant arthritis	days *total crocins.	↓ joint damage	2019)
Rats with metabolic syndrome □induced osteoporosis	5 or 10 mg/kg/day orally, 5 days/week for 12 weeks	Crocin 10 mg/kg ↓ elevated IL□6 and TNF□α in serum.	(Algandaby 2019)
Macrophage/monocyte progenitors isolated from mouse bone marrow	0-200 μM	↓ RANKL-induced NF-κB and NFATc1 activation	(Fu, Pan et al. 2017)

Abbreviations: IL: Interleukin; TNF: Tumor necrosis factor; iNOS: Inducible nitric oxide synthase; LPS: Lipopolysaccharide; NF-κB: Nuclear Factor-kappa B; MMP: Matrix metalloprotease; JNK: c-Jun N-terminal Kinase; TGF: Transforming growth factor; RANKL: receptor activator of nuclear factor-kappaB ligand.

Acknowledgments: The authors would like to thank Mrs. D. Pantartzi, Scientific Secretary of the Clinical Trial Office of the Department of Urology, University of Crete, Medical School, Heraklion, Crete, Greece, for the administrative and technical support.

Funding:

Authors thank the vice chancellor for research, Mashhad University of Medical Sciences (MUMS) for the financial support. This work was approved by the Ethics committee of MUMS (approval No. IR.MUMS.MEDICAL.REC.1397.573) and completed/submitted by Elham Shojaei Asrami as the thesis to fulfill MD degree.

Conflicts of Interest:

Authors declare no conflicts of interest.

References

Abou-Hany, H. O., H. Atef, E. Said, H. A. Elkashef and H. A. Salem (2018). "Crocin mediated amelioration of oxidative burden and inflammatory cascade suppresses diabetic nephropathy progression in diabetic rats." Chemico-biological interactions **284**: 90-100.

Abou-Hany, H. O., H. Atef, E. Said, H. A. Elkashef and H. A. Salem (2018). "Crocin reverses unilateral renal ischemia reperfusion injury-induced augmentation of oxidative stress and toll like receptor-4 activity." Environmental toxicology and pharmacology **59**: 182-189.

Adali, F., Y. Gonul, M. Aldemir, O. Hazman, A. Ahsen, M. F. Bozkurt, O. G. Sen, I. Keles and H. Keles (2016). "Investigation of the effect of crocin pretreatment on renal injury induced by infrarenal aortic occlusion." Journal of Surgical Research **203**(1): 145-153.

Akira, S. and T. Kishimoto (1996). "Role of interleukin-6 in macrophage function." Current opinion in hematology **3**(1): 87-93.

Alavizadeh, S. H. and H. Hosseinzadeh (2014). "Bioactivity assessment and toxicity of crocin: a comprehensive review." Food and Chemical Toxicology **64**: 65-80.

Algandaby, M. M. (2018). "Antifibrotic effects of crocin on thioacetamide-induced liver fibrosis in mice." Saudi journal of biological sciences **25**(4): 747-754.

Algandaby, M. M. (2019). "Crocin attenuates metabolic syndrome□induced osteoporosis in rats." Journal of food biochemistry **43**(7): e12895.

Altinoz, E., Z. Oner, H. Elbe, Y. Cigremis and Y. Turkoz (2015). "Protective effects of saffron (its active constituent, crocin) on nephropathy in streptozotocin-induced diabetic rats." Human & experimental toxicology **34**(2): 127-134.

Amerizadeh, F., N. Rezaei, F. Rahmani, S. M. Hassanian, R. Moradi□Marjaneh, H. Fiuji, N. Boroumand, A. Nosrati□Tirkani, M. Ghayour□Mobarhan and G. A. Ferns (2018). "Crocin

829 synergistically enhances the antiproliferative activity of 5□flurouracil through Wnt/PI3K
830 pathway in a mouse model of colitis□associated colorectal cancer." Journal of cellular
831 biochemistry **119**(12): 10250-10261.
832 Amin, A., A. A Hamza, S. Daoud, K. Khazanehdari, A. Al Hrout, B. Baig, A. Chaiboonchoe, T.
833 E Adrian, N. Zaki and K. Salehi-Ashtiani (2016). "Saffron-based crocin prevents early lesions of
834 liver cancer: in vivo, in vitro and network analyses." Recent patents on anti-cancer drug
835 discovery **11**(1): 121-133.
836 Amin, B. and H. Hosseinzadeh (2012). "Evaluation of aqueous and ethanolic extracts of saffron,
837 Crocus sativus L., and its constituents, safranal and crocin in allodynia and hyperalgesia induced
838 by chronic constriction injury model of neuropathic pain in rats." Fitoterapia **83**(5): 888-895.
839 Avruch, J. (2007). "MAP kinase pathways: the first twenty years." Biochimica et Biophysica
840 Acta (BBA)-Molecular Cell Research **1773**(8): 1150-1160.
841 Bahashwan, S., M. H. Hassan, H. Aly, M. M. Ghobara, H. A. El-Beshbishy and I. Busati (2015).
842 "Crocin mitigates carbon tetrachloride-induced liver toxicity in rats." Journal of Taibah
843 university medical sciences **10**(2): 140-149.
844 Baldin, V., J. Lukas, M. J. Marcote, M. Pagano and G. Draetta (1993). "Cyclin D1 is a nuclear
845 protein required for cell cycle progression in G1." Genes & development **7**(5): 812-821.
846 Bandiera, S., S. Pfeffer, T. F. Baumert and M. B. Zeisel (2015). "miR-122–a key factor and
847 therapeutic target in liver disease." Journal of hepatology **62**(2): 448-457.
848 Berenbaum, F. (2004). "Signaling transduction: target in osteoarthritis." Current opinion in
849 rheumatology **16**(5): 616-622.
850 Blumberg, R. S. (2009). "Inflammation in the intestinal tract: pathogenesis and treatment."
851 Digestive diseases **27**(4): 455-464.

852 Bosco, A., C. O. Romero, K. T. Breen, A. A. Chagovetz, M. R. Steele, B. K. Ambati and M. L.
853 Vetter (2015). "Neurodegeneration severity can be predicted from early microglia alterations
854 monitored in vivo in a mouse model of chronic glaucoma." Disease models & mechanisms **8**(5):
855 443-455.
856 Bosco, A., M. R. Steele and M. L. Vetter (2011). "Early microglia activation in a mouse model
857 of chronic glaucoma." Journal of Comparative Neurology **519**(4): 599-620.
858 Bowie, A. and L. A. O'Neill (2000). "Oxidative stress and nuclear factor-κB activation: a
859 reassessment of the evidence in the light of recent discoveries." Biochemical pharmacology
860 **59**(1): 13-23.
861 Cargnello, M. and P. P. Roux (2011). "Activation and function of the MAPKs and their
862 substrates, the MAPK-activated protein kinases." Microbiol. Mol. Biol. Rev. **75**(1): 50-83.
863 Carlezon Jr, W. A., R. S. Duman and E. J. Nestler (2005). "The many faces of CREB." Trends in
864 neurosciences **28**(8): 436-445.
865 Carrasco, C., M. Naziroğlu, A. B. Rodríguez and J. A. Pariente (2018). "Neuropathic pain:
866 delving into the oxidative origin and the possible implication of transient receptor potential
867 channels." Frontiers in physiology **9**: 95.
868 Chiu, J. and I. W. Dawes (2012). "Redox control of cell proliferation." Trends in cell biology
869 **22**(11): 592-601.
870 Chong, Z. Z. (2016). "Targeting PRAS40 for multiple diseases." Drug discovery today **21**(8):
871 1222-1231.
872 Cosgun, B. E., M. Erdemli, M. Gul, S. Gul, H. G. Bag, Z. Aksungur and E. Altinoz (2018).
873 "Crocin protects intestine tissue against carbon tetrachloride-mediated oxidative stress in rats."
874 Gen Physiol Biophys **37**: 399-409.

de Sousa, L. M., J. M. dos Santos Alves, C. da Silva Martins, K. M. A. Pereira, P. Goes and D. V. Gondim (2019). "Immunoexpression of canonical Wnt and NF-κB signaling pathways in the temporomandibular joint of arthritic rats." Inflammation Research **68**(10): 889-900.

Demoruelle, M. K., K. D. Deane and V. M. Holers (2014). "When and where does inflammation begin in rheumatoid arthritis?" Current opinion in rheumatology **26**(1): 64.

Deslauriers, A. M., A. Afkhami-Goli, A. M. Paul, R. K. Bhat, S. Acharjee, K. K. Ellestad, F. Noorbakhsh, M. Michalak and C. Power (2011). "Neuroinflammation and endoplasmic reticulum stress are coregulated by crocin to prevent demyelination and neurodegeneration." The Journal of Immunology **187**(9): 4788-4799.

Dianat, M., M. Radan, M. Badavi, S. A. Mard, V. Bayati and M. Ahmadizadeh (2018). "Crocin attenuates cigarette smoke-induced lung injury and cardiac dysfunction by anti-oxidative effects: the role of Nrf2 antioxidant system in preventing oxidative stress." Respiratory research **19**(1): 58.

Diehl, J. A., M. Cheng, M. F. Roussel and C. J. Sherr (1998). "Glycogen synthase kinase-3β regulates cyclin D1 proteolysis and subcellular localization." Genes & development **12**(22): 3499-3511.

Dinarello, C. A. (2004). "Interleukin-18 and the treatment of rheumatoid arthritis." Rheumatic diseases clinics of North America **30**(2): 417-434, ix.

Ding, Q., H. Zhong, Y. Qi, Y. Cheng, W. Li, S. Yan and X. Wang (2013). "Anti-arthritic effects of crocin in interleukin-1β-treated articular chondrocytes and cartilage in a rabbit osteoarthritic model." Inflammation Research **62**(1): 17-25.

Docea, A. O., E. Gofita, M. Goumenou, D. Calina, O. Rogoveanu, M. Varut, C. Olaru, E. Kerasioti, P. Fountoucidou and I. Taitzoglou (2018). "Six months exposure to a real life mixture

of 13 chemicals' below individual NOAELs induced non monotonic sex-dependent biochemical and redox status changes in rats." Food and chemical toxicology **115**: 470-481.

Dowlati, Y., N. Herrmann, W. Swardfager, H. Liu, L. Sham, E. K. Reim and K. L. Lanctôt (2010). "A meta-analysis of cytokines in major depression." Biological psychiatry **67**(5): 446-457.

Du, J., Y. Chi, Z. Song, Q. Di, Z. Mai, J. Shi and M. Li (2018). "Crocin reduces Aspergillus fumigatus□induced airway inflammation and NF□κB signal activation." Journal of cellular biochemistry **119**(2): 1746-1754.

Duran-Salgado, M. B. and A. F. Rubio-Guerra (2014). "Diabetic nephropathy and inflammation." World journal of diabetes **5**(3): 393.

Ebrahimzadeh, A., S. Y. Moghadam, H. Rahimi, M. Motaghinejad, M. Motevalian, S. Safari and M. A. Mesrabadi (2019). "Crocin acts as a neuroprotective mediator against methylphenidate□induced neurobehavioral and neurochemical sequelae: Possible role of the CREB□BDNF signaling pathway." Acta Neurobiologiae Experimentalis **79**(4).

El-Fawal, R., H. M. El Fayoumi and M. F. Mahmoud (2018). "Diosmin and crocin alleviate nephropathy in metabolic syndrome rat model: Effect on oxidative stress and low grade inflammation." Biomedicine & Pharmacotherapy **102**: 930-937.

El-Fawal, R., H. M. El Fayoumi and M. F. Mahmoud (2019). "Effects of diosmin and crocin on metabolic syndrome-associated cardio-vascular complications in rats." Naunyn-Schmiedeberg's archives of pharmacology **392**(12): 1523-1536.

El-Maraghy, S. A., S. M. Rizk and N. N. Shahin (2015). "Gastroprotective effect of crocin in ethanol-induced gastric injury in rats." Chemico-biological interactions **229**: 26-35.

Elsherbiny, N. M., M. F. Salama, E. Said, M. El-Sherbiny and M. M. Al-Gayyar (2016). "Crocin protects against doxorubicin-induced myocardial toxicity in rats through down-regulation of inflammatory and apoptic pathways." Chemico-biological interactions **247**: 39-48.

Emamian, E. (2012). "AKT/GSK3 signaling pathway and schizophrenia." Frontiers in molecular neuroscience **5**: 33.

Erben-Russ, M., C. Michel, W. Bors and M. Saran (1987). "The reaction of sulfite radical anion with nucleic acid components." Free radical research communications **2**(4-6): 285-288.

Erdemli, M. E., M. Gul, E. Altinoz, Z. Aksungur, S. Gul and H. G. Bag (2018). "Can crocin play a preventive role in Wistar rats with carbon tetrachloride-induced nephrotoxicity?" Iranian journal of basic medical sciences **21**(4): 382.

Erdemli, M. E., M. Gul, E. Altinoz, E. Zayman, Z. Aksungur and H. G. Bag (2017). "The protective role of crocin in tartrazine induced nephrotoxicity in Wistar rats." Biomedicine & Pharmacotherapy **96**: 930-935.

Erfanparast, A., E. Tamaddonfard, M. Taati and M. Dabbaghi (2015). "Effects of crocin and safranal, saffron constituents, on the formalin-induced orofacial pain in rats." Avicenna journal of phytomedicine **5**(5): 392.

Fagot, D., D. Pham, J. Laboureau, E. Planel, L. Guerin, C. Nègre, M. Donovan and B. Bernard (2018). "Crocin, a natural molecule with potentially beneficial effects against skin ageing." International journal of cosmetic science **40**(4): 388-400.

Faramarzpour, A., A. A. Tehrani, E. Tamaddonfard and M. Imani (2019). The effects of crocin, mesalazine and their combination in the acetic acid-induced colitis in rats. Veterinary Research Forum, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Farshid, A. A. and E. Tamaddonfard (2015). "Histopathological and behavioral evaluations of the effects of crocin, safranal and insulin on diabetic peripheral neuropathy in rats." Avicenna journal of phytomedicine **5**(5): 469.

Felix, R. S., G. W. Colleoni, O. L. Caballero, M. Yamamoto, M. S. Almeida, V. C. Andrade, L. Maria de Lourdes, W. A. da Silva Jr, M. D. Begnami and F. A. Soares (2009). "SAGE analysis highlights the importance of p53csv, ddx5, mapkapk2 and ranbp2 to multiple myeloma tumorigenesis." Cancer letters **278**(1): 41-48.

Fountoucidou, P., A. S. Veskoukis, E. Kerasioti, A. O. Docea, I. A. Taitzoglou, J. Liesivuori, A. Tsatsakis and D. Kouretas (2019). "A mixture of routinely encountered xenobiotics induces both redox adaptations and perturbations in blood and tissues of rats after a long-term low-dose exposure regimen: The time and dose issue." Toxicology letters **317**: 24-44.

Fu, L., F. Pan and Y. Jiao (2017). "Crocin inhibits RANKL-induced osteoclast formation and bone resorption by suppressing NF-κB signaling pathway activation." Immunobiology **222**(4): 597-603.

Gedik, S., M. E. Erdemli, M. Gul, B. Yigitcan, H. G. Bag, Z. Aksungur and E. Altinoz (2017). "Hepatoprotective effects of crocin on biochemical and histopathological alterations following acrylamide-induced liver injury in Wistar rats." Biomedicine & pharmacotherapy **95**: 764-770.

Ghiasian, M., F. Khamisabadi, N. Kheiripour, M. Karami, R. Haddadi, A. Ghaleiha, B. Taghvaei, S. S. Oliaie, M. Salehi and P. Samadi (2019). "Effects of crocin in reducing DNA damage, inflammation, and oxidative stress in multiple sclerosis patients: A double□blind, randomized, and placebo□controlled trial." Journal of Biochemical and Molecular Toxicology **33**(12): e22410.

964 Giebelen, I. A., D. J. van Westerloo, G. J. LaRosa, A. F. de Vos and T. van der Poll (2007).
965 "Local stimulation of α7 cholinergic receptors inhibits LPS-induced TNF-α release in the mouse
966 lung." Shock **28**(6): 700-703.
967 Godugu, C., L. P. Pasari, A. Khurana, P. Anchi, M. A. Saifi, S. P. Bansod and S. Annaldas
968 (2019). "Crocin, an active constituent of Crocus sativus ameliorates cerulein induced pancreatic
969 inflammation and oxidative stress." Phytotherapy Research.
970 Goyal, S., S. Arora, A. Sharma, S. Joshi, R. Ray, J. Bhatia, S. Kumari and D. Arya (2010).
971 "Preventive effect of crocin of Crocus sativus on hemodynamic, biochemical, histopathological
972 and ultrastuctural alterations in isoproterenol-induced cardiotoxicity in rats." Phytomedicine
973 **17**(3-4): 227-232.
974 Graeber, M. B. and W. J. Streit (2010). "Microglia: biology and pathology." Acta
975 neuropathologica **119**(1): 89-105.
976 Grünig, G., M. Warnock, A. E. Wakil, R. Venkayya, F. Brombacher, D. M. Rennick, D.
977 Sheppard, M. Mohrs, D. D. Donaldson and R. M. Locksley (1998). "Requirement for IL-13
978 independently of IL-4 in experimental asthma." Science **282**(5397): 2261-2263.
979 Hamaguchi, M., T. Watanabe, K. Higuchi, K. Tominaga, Y. Fujiwara and T. Arakawa (2001).
980 "Mechanisms and roles of neutrophil infiltration in stress-induced gastric injury in rats."
981 Digestive diseases and sciences **46**(12): 2708-2715.
982 Hashemzaei, M., M. Abdollahzadeh, M. Iranshahi, E. Golmakani, R. Rezaee and K. Tabrizian
983 (2017). "Effects of luteolin and luteolin-morphine co-administration on acute and chronic pain
984 and sciatic nerve ligated-induced neuropathy in mice." Journal of Complementary and
985 Integrative Medicine **14**(1).

Hashemzaei, M., A. Delarami Far, A. Yari, R. E. Heravi, K. Tabrizian, S. M. Taghdisi, S. E. Sadegh, K. Tsarouhas, D. Kouretas and G. Tzanakakis (2017). "Anticancer and apoptosis□inducing effects of quercetin in vitro and in vivo." Oncology reports **38**(2): 819-828.

Hashemzaei, M., M. Imen Shahidi, S. A. Moallem, K. Abnous, M. Ghorbani and A. H. Mohamadpour (2016). "Modulation of JAK2, STAT3 and Akt1 proteins by granulocyte colony stimulating factor following carbon monoxide poisoning in male rat." Drug and chemical toxicology **39**(4): 375-379.

Hashemzaei, M., R. Rezaee, M. Nabatzehi, K. Tsarouhas, T. K. Nikolouzakis, G. Lazopoulos, D. A. Spandidos, A. Tsatsakis and J. Shahraki "Anti□hypertensive effect of crocin and hesperidin combination in high□fat diet treated rats." Experimental and Therapeutic Medicine.

Hashemzaei, M., M. A. SadeghiBonjar, K. Tabrizian, M. Iranshahi, M. Iranshahy and R. Rezaee (2015). "Evaluation of the analgesic effect of Umbelliprenin and Umbelliprenin-morphine co-administration on the acute, chronic and neuropathic pain." Indian J Pharm Educ **49**(2): 121-125.

Hassan, M. H., S. A. Bahashawan, T. M. Abdelghany, G. M. Abd□Allah and M. M. Ghobara (2015). "Crocin Abrogates Carbon Tetrachloride□Induced Renal Toxicity in Rats via Modulation of Metabolizing Enzymes and Diminution of Oxidative Stress, Apoptosis, and Inflammatory Cytokines." Journal of biochemical and molecular toxicology **29**(7): 330-339.

Hassani, F. V., S. Mehri, K. Abnous, R. Birner-Gruenberger and H. Hosseinzadeh (2017). "Protective effect of crocin on BPA-induced liver toxicity in rats through inhibition of oxidative stress and downregulation of MAPK and MAPKAP signaling pathway and miRNA-122 expression." Food and Chemical Toxicology **107**: 395-405.

1007 Hassani, F. V., R. Rezaee, H. Sazegara, M. Hashemzaei, K. Shirani and G. Karimi (2015).
1008 "Effects of silymarin on neuropathic pain and formalin-induced nociception in mice." Iranian
1009 journal of basic medical sciences **18**(7): 715.
1010 Hatziagapiou, K., E. Kakouri, G. I. Lambrou, K. Bethanis and P. A. Tarantilis (2019).
1011 "Antioxidant properties of Crocus sativus L. and its constituents and relevance to
1012 neurodegenerative diseases; focus on Alzheimer's and Parkinson's disease." Current
1013 neuropharmacology **17**(4): 377-402.
1014 HAZMAN, Ö., L. Aksoy and A. BÜYÜKBEN (2016). "Effects of crocin on experimental
1015 obesity and type-2 diabetes." Turkish journal of medical sciences **46**(5): 1593-1602.
1016 Heidari, S., S. Mehri and H. Hosseinzadeh (2017). "Memory enhancement and protective effects
1017 of crocin against D-galactose aging model in the hippocampus of Wistar rats." Iranian journal of
1018 basic medical sciences **20**(11): 1250.
1019 Hemshekhar, M., M. S. Santhosh, K. Sunitha, R. Thushara, K. Kemparaju, K. Rangappa and K.
1020 Girish (2012). "A dietary colorant crocin mitigates arthritis and associated secondary
1021 complications by modulating cartilage deteriorating enzymes, inflammatory mediators and
1022 antioxidant status." Biochimie **94**(12): 2723-2733.
1023 Hermida, M. A., J. D. Kumar and N. R. Leslie (2017). "GSK3 and its interactions with the
1024 PI3K/AKT/mTOR signalling network." Advances in biological regulation **65**: 5-15.
1025 Hinz, M., D. Krappmann, A. Eichten, A. Heder, C. Scheidereit and M. Strauss (1999). "NF-κB
1026 function in growth control: regulation of cyclin D1 expression and G0/G1-to-S-phase transition."
1027 Molecular and cellular biology **19**(4): 2690-2698.

1028 Hobbenaghi, R., S. Ghafarzadeh, E. Tamaddonfard, A. A. Farshid and M. Imani (2019). Crocin
1029 exerts improving effects on indomethacin-induced small intestinal ulcer by antioxidant, anti-
1030 inflammatory and anti-apoptotic mechanisms. Veterinary Research Forum.
1031 Hu, Y., X. Liu, Q. Xia, T. Yin, C. Bai, Z. Wang, L. Du, X. Li, W. Wang and L. Sun (2019).
1032 "Comparative anti-arthritic investigation of iridoid glycosides and crocetin derivatives from
1033 Gardenia jasminoides Ellis in Freund's complete adjuvant-induced arthritis in rats."
1034 Phytomedicine **53**: 223-233.
1035 Huang, A. and L. Jia (2019). "Crocin enhances hypothermia therapy in hypoxic ischemia-
1036 induced brain injury in mice." Acta Neurologica Belgica: 1-8.
1037 Huang, Z., J. Xu, X. Huang, G. Sun, R. Jiang, H. Wu, X. Shan, K. Bao, Q. Wu and H. Wu
1038 (2019). "Crocin induces anti-ischemia in middle cerebral artery occlusion rats and inhibits
1039 autophagy by regulating the mammalian target of rapamycin." European journal of
1040 pharmacology **857**: 172424.
1041 Iwasa, R., A. Yamada, K. Sono, R. Furukawa, K. Takeuchi and Y. Suzuki (2015). "C-reactive
1042 protein level at 2 weeks following initiation of infliximab induction therapy predicts outcomes in
1043 patients with ulcerative colitis: a 3 year follow-up study." BMC gastroenterology **15**(1): 103.
1044 Jiang, Q., M. Yi, Q. Guo, C. Wang, H. Wang, S. Meng, C. Liu, Y. Fu, H. Ji and T. Chen (2015).
1045 "Protective effects of polydatin on lipopolysaccharide-induced acute lung injury through TLR4-
1046 MyD88-NF-κB pathway." International immunopharmacology **29**(2): 370-376.
1047 Jin, W., Y. Zhang, Y. Xue, X. Han, X. Zhang, Z. Ma, S. Sun, X. Chu, J. Cheng and S. Guan
1048 (2020). "Crocin attenuates isoprenaline-induced myocardial fibrosis by targeting TLR4/NF-κB
1049 signaling: connecting oxidative stress, inflammation, and apoptosis." Naunyn-Schmiedeberg's
1050 Archives of Pharmacology **393**(1): 13-23.

1051 Jnaneshwari, S., M. Hemshekhar, M. S. Santhosh, K. Sunitha, R. Thushara, C.
1052 Thirunavukkarasu, K. Kemparaju and K. S. Girish (2013). "C rocin, a dietary colorant mitigates
1053 cyclophosphamide□induced organ toxicity by modulating antioxidant status and inflammatory
1054 cytokines." Journal of Pharmacy and Pharmacology **65**(4): 604-614.
1055 Jope, R. S., C. J. Yuskaitis and E. Beurel (2007). "Glycogen synthase kinase-3 (GSK3):
1056 inflammation, diseases, and therapeutics." Neurochemical research **32**(4-5): 577-595.
1057 Kalantar, M., H. Kalantari, M. Goudarzi, L. Khorsandi, S. Bakhit and H. Kalantar (2019).
1058 "Crocin ameliorates methotrexate-induced liver injury via inhibition of oxidative stress and
1059 inflammation in rats." Pharmacological Reports **71**(4): 746-752.
1060 Karami, M., S. Z. Bathaie, T. Tiraihi, M. Habibi-Rezaei, J. Arabkheradmand and S. Faghihzadeh
1061 (2013). "Crocin improved locomotor function and mechanical behavior in the rat model of
1062 contused spinal cord injury through decreasing calcitonin gene related peptide (CGRP)."
1063 Phytomedicine **21**(1): 62-67.
1064 Kavasi, R.-M., A. Berdiaki, I. Spyridaki, E. Corsini, A. Tsatsakis, G. Tzanakakis and D.
1065 Nikitovic (2017). "HA metabolism in skin homeostasis and inflammatory disease." Food and
1066 chemical toxicology **101**: 128-138.
1067 Kawabata, K., N. H. Tung, Y. Shoyama, S. Sugie, T. Mori and T. Tanaka (2012). "Dietary crocin
1068 inhibits colitis and colitis-associated colorectal carcinogenesis in male ICR mice." Evidence-
1069 based complementary and alternative medicine **2012**.
1070 Khodir, A. E., E. Said, H. Atif, H. A. ElKashef and H. A. Salem (2019). "Targeting Nrf2/HO-1
1071 signaling by crocin: Role in attenuation of AA-induced ulcerative colitis in rats." Biomedicine &
1072 Pharmacotherapy **110**: 389-399.

1073 Khor, T. O., M.-T. Huang, K. H. Kwon, J. Y. Chan, B. S. Reddy and A.-N. Kong (2006). "Nrf2-
1074 deficient mice have an increased susceptibility to dextran sulfate sodium–induced colitis."
1075 Cancer research **66**(24): 11580-11584.
1076 Ki, Y.-W., J. H. Park, J. E. Lee, I. C. Shin and H. C. Koh (2013). "JNK and p38 MAPK regulate
1077 oxidative stress and the inflammatory response in chlorpyrifos-induced apoptosis." Toxicology
1078 letters **218**(3): 235-245.
1079 Kim, J.-H., G.-Y. Park, S. Y. Bang, S. Y. Park, S.-K. Bae and Y. Kim (2014). "Crocin
1080 suppresses LPS-stimulated expression of inducible nitric oxide synthase by upregulation of heme
1081 oxygenase-1 via calcium/calmodulin-dependent protein kinase 4." Mediators of inflammation
1082 **2014**.
1083 Kim, W.-H., H.-J. An, J.-Y. Kim, M.-G. Gwon, H. Gu, S.-J. Lee, J. Y. Park, K.-D. Park, S.-M.
1084 Han and M.-K. Kim (2017). "Apamin inhibits TNF-α-and IFN-γ-induced inflammatory
1085 cytokines and chemokines via suppressions of NF-κB signaling pathway and STAT in human
1086 keratinocytes." Pharmacological Reports **69**(5): 1030-1035.
1087 Kocaman, G., E. Altinoz, M. Erdemli, M. Gul, Z. Erdemli, S. Gul and H. Bag (2019). "Protective
1088 effects of crocin on biochemistry and histopathology of experimental periodontitis in rats."
1089 Biotechnic & Histochemistry **94**(5): 366-373.
1090 Korani, S., M. Korani, T. Sathyapalan and A. Sahebkar (2019). "Therapeutic effects of Crocin in
1091 autoimmune diseases: A review." BioFactors **45**(6): 835-843.
1092 Kotlyarov, A., A. Neininger, C. Schubert, R. Eckert, C. Birchmeier, H.-D. Volk and M. Gaestel
1093 (1999). "MAPKAP kinase 2 is essential for LPS-induced TNF-α biosynthesis." Nature cell
1094 biology **1**(2): 94-97.

1095 Koyama, Y. and D. A. Brenner (2017). "Liver inflammation and fibrosis." The Journal of clinical
1096 investigation **127**(1): 55-64.
1097 Kuprash, D. and S. Nedospasov (2016). "Molecular and cellular mechanisms of inflammation."
1098 Biochemistry (Moscow) **81**(11): 1237-1239.
1099 Laplante, M. and D. M. Sabatini (2012). "mTOR signaling in growth control and disease." Cell
1100 **149**(2): 274-293.
1101 Lawan, A., L. Zhang, F. Gatzke, K. Min, M. J. Jurczak, M. Al-Mutairi, P. Richter, J. P. G.
1102 Camporez, A. Couvillon and D. Pesta (2015). "Hepatic mitogen-activated protein kinase
1103 phosphatase 1 selectively regulates glucose metabolism and energy homeostasis." Molecular and
1104 cellular biology **35**(1): 26-40.
1105 Lei, M., C. Guo, L. Hua, S. Xue, D. Yu, C. Zhang and D. Wang (2017). "Crocin attenuates joint
1106 pain and muscle dysfunction in osteoarthritis rat." Inflammation **40**(6): 2086-2093.
1107 Leonard, B. E., D. McCartan, J. White and D. J. King (2004). "Methylphenidate: a review of its
1108 neuropharmacological, neuropsychological and adverse clinical effects." Human
1109 Psychopharmacology: Clinical and Experimental **19**(3): 151-180.
1110 Li, J., H.-t. Lei, L. Cao, Y.-N. Mi, S. Li and Y.-X. Cao (2018). "Crocin alleviates coronary
1111 atherosclerosis via inhibiting lipid synthesis and inducing M2 macrophage polarization."
1112 International immunopharmacology **55**: 120-127.
1113 Li, K., Y. Li, J. Mi, X. Han and J. Zhao (2016). "Intradiscal injection of crocin retards lesion
1114 induced intervertebral disc degeneration." Int J Clin Exp Pathol **9**(4): 4833-4842.
1115 Li, L., H. Zhang, S. Jin and C. Liu (2018). "Effects of crocin on inflammatory activities in
1116 human fibroblast-like synoviocytes and collagen-induced arthritis in mice." Immunologic
1117 research **66**(3): 406-413.

1118 Li, N. and M. KARIN (1999). "Is NF-κB the sensor of oxidative stress?" The FASEB Journal
1119 **13**(10): 1137-1143.
1120 Li, Q. and I. M. Verma (2002). "NF-κB regulation in the immune system." Nature Reviews
1121 Immunology **2**(10): 725-734.
1122 Li, S., X. Liu, J. Lei, J. Yang, P. Tian and Y. Gao (2017). "Crocin protects podocytes against
1123 oxidative stress and inflammation induced by high glucose through inhibition of NF-κB."
1124 Cellular Physiology and Biochemistry **42**(4): 1481-1492.
1125 Li, X., C. Fan, Z. Xiao, Y. Zhao, H. Zhang, J. Sun, Y. Zhuang, X. Wu, J. Shi and Y. Chen
1126 (2018). "A collagen microchannel scaffold carrying paclitaxel-liposomes induces neuronal
1127 differentiation of neural stem cells through Wnt/β-catenin signaling for spinal cord injury repair."
1128 Biomaterials **183**: 114-127.
1129 Liang, J. J., H. R. Li, Y. Chen, C. Zhang, Z. C. Liang, Y. Q. Shi, L. L. Zhang, L. Xin and D. B.
1130 Zhao (2019). "Diallyl Trisulfide can induce fibroblast-like synovial apoptosis and has a
1131 therapeutic effect on collagen-induced arthritis in mice via blocking NF-κB and Wnt pathways."
1132 International immunopharmacology **71**: 132-138.
1133 Lim, H., K.-R. Park, D.-U. Lee, Y.-S. Kim and H.-P. Kim (2008). "Effects of the constituents of
1134 gardenia fructus on prostaglandin and NO production." Biomolecules & Therapeutics **16**(2): 82-
1135 86.
1136 Liu, B., L. Yang, B. Huang, M. Cheng, H. Wang, Y. Li, D. Huang, J. Zheng, Q. Li and X. Zhang
1137 (2012). "A functional copy-number variation in MAPKAPK2 predicts risk and prognosis of lung
1138 cancer." The American Journal of Human Genetics **91**(2): 384-390.

1139 Liu, W., Y. Sun, Z. Cheng, Y. Guo, P. Liu and Y. Wen (2018). "Crocin exerts anti-inflammatory
1140 and anti-arthritic effects on type II collagen-induced arthritis in rats." Pharmaceutical biology
1141 **56**(1): 209-216.
1142 Liu, Y., X. Qin and X. Lu (2018). "Crocin improves endometriosis by inhibiting cell
1143 proliferation and the release of inflammatory factors." Biomedicine & Pharmacotherapy **106**:
1144 1678-1685.
1145 Loboda, A., M. Damulewicz, E. Pyza, A. Jozkowicz and J. Dulak (2016). "Role of Nrf2/HO-1
1146 system in development, oxidative stress response and diseases: an evolutionarily conserved
1147 mechanism." Cellular and molecular life sciences **73**(17): 3221-3247.
1148 Luo, L., K. Fang, X. Dan and M. Gu (2019). "Crocin ameliorates hepatic steatosis through
1149 activation of AMPK signaling in db/db mice." Lipids in health and disease **18**(1): 1-9.
1150 Lv, B., F. Huo, Z. Zhu, Z. Xu, X. Dang, T. Chen, T. Zhang and X. Yang (2016). "Crocin
1151 upregulates CX3CR1 expression by suppressing NF-κB/YY1 signaling and inhibiting
1152 lipopolysaccharide-induced microglial activation." Neurochemical research **41**(8): 1949-1957.
1153 Malutan, A. M., T. Drugan, N. Costin, R. Ciortea, C. Bucuri, M. P. Rada and D. Mihu (2015).
1154 "Pro-inflammatory cytokines for evaluation of inflammatory status in endometriosis." Central-
1155 European journal of immunology **40**(1): 96.
1156 Mard, S. A., Z. Nikraftar, Y. Farbood and E. Mansouri (2015). "A preliminary study of the anti-
1157 inflammatory and anti-apoptotic effects of crocin against gastric ischemia-reperfusion injury in
1158 rats." Brazilian Journal of Pharmaceutical Sciences **51**(3): 637-642.
1159 Mard, S. A., M. H. Pipelzadeh, A. Teimoori, N. Neisi, S. Mojahedin, M. Z. S. Khani and I.
1160 Ahmadi (2016). "Protective activity of crocin against indomethacin-induced gastric lesions in
1161 rats." Journal of natural medicines **70**(1): 62-74.

1162 Martinowich, K., D. Hattori, H. Wu, S. Fouse, F. He, Y. Hu, G. Fan and Y. E. Sun (2003).
1163 "DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation."
1164 Science **302**(5646): 890-893.
1165 Mayr, B. and M. Montminy (2001). "Transcriptional regulation by the phosphorylation-
1166 dependent factor CREB." Nature reviews Molecular cell biology **2**(8): 599-609.
1167 Mofidi, R., M. Duff, S. Wigmore, K. Madhavan, O. Garden and R. Parks (2006). "Association
1168 between early systemic inflammatory response, severity of multiorgan dysfunction and death in
1169 acute pancreatitis." British Journal of Surgery: Incorporating European Journal of Surgery and
1170 Swiss Surgery **93**(6): 738-744.
1171 Mohammadi, E., S. Mehri, H. B. Bostan and H. Hosseinzadeh (2018). "Protective effect of
1172 crocin against d-galactose-induced aging in mice." Avicenna journal of phytomedicine **8**(1): 14.
1173 Mohammadzadeh, L., K. Abnous, B. M. Razavi and H. Hosseinzadeh (2020). "Crocin-protected
1174 malathion-induced spatial memory deficits by inhibiting TAU protein hyperphosphorylation and
1175 antiapoptotic effects." Nutritional Neuroscience **23**(3): 221-236.
1176 Mohammadzadeh, L., H. Hosseinzadeh, K. Abnous and B. M. Razavi (2018). "Neuroprotective
1177 potential of crocin against malathion-induced motor deficit and neurochemical alterations in
1178 rats." Environmental Science and Pollution Research **25**(5): 4904-4914.
1179 Moher, D., A. Liberati, J. Tetzlaff, D. G. Altman and P. Group (2010). "Preferred reporting items
1180 for systematic reviews and meta-analyses: the PRISMA statement." Int J Surg **8**(5): 336-341.
1181 Molinos, M., C. R. Almeida, J. Caldeira, C. Cunha, R. M. Gonçalves and M. A. Barbosa (2015).
1182 "Inflammation in intervertebral disc degeneration and regeneration." Journal of the Royal
1183 Society Interface **12**(104): 20141191.

1184 Morel, J. C., C. C. Park, K. Zhu, P. Kumar, J. H. Ruth and A. E. Koch (2002). "Signal
1185 transduction pathways involved in rheumatoid arthritis synovial fibroblast interleukin-18-
1186 induced vascular cell adhesion molecule-1 expression." Journal of Biological Chemistry **277**(38):
1187 34679-34691.
1188 Morton, W. A. and G. G. Stockton (2000). "Methylphenidate abuse and psychiatric side effects."
1189 Primary care companion to the Journal of clinical psychiatry **2**(5): 159.
1190 Motaghinejad, M., S. Safari, S. Feizipour and S. Sadr (2019). "Crocin may be useful to prevent
1191 or treatment of alcohol induced neurodegeneration and neurobehavioral sequels via modulation
1192 of CREB/BDNF and Akt/GSK signaling pathway." Medical hypotheses **124**: 21-25.
1193 Mozaffari, S., S. R. Yasuj, M. Motaghinejad, M. Motevalian and R. Kheiri (2019). "Crocin
1194 Acting as a Neuroprotective Agent against Methamphetamine-induced Neurodegeneration via
1195 CREB-BDNF Signaling Pathway." Iranian journal of pharmaceutical research: IJPR **18**(2): 745.
1196 Nam, K. N., Y.-M. Park, H.-J. Jung, J. Y. Lee, B. D. Min, S.-U. Park, W.-S. Jung, K.-H. Cho, J.-
1197 H. Park and I. Kang (2010). "Anti-inflammatory effects of crocin and crocetin in rat brain
1198 microglial cells." European journal of pharmacology **648**(1-3): 110-116.
1199 Neagu, M., C. Constantin, I. D. Popescu, D. Zipeto, G. N. Tzanakakis, D. Nikitovic, C. Fenga, C.
1200 Stratakis, D. A. Spandidos and A. M. Tsatsakis (2019). "Inflammation and metabolism in cancer
1201 cell–mitochondria key player." Frontiers in oncology **9**: 348.
1202 Omoigui, S. (2007). "The biochemical origin of pain: the origin of all pain is inflammation and
1203 the inflammatory response. Part 2 of 3–inflammatory profile of pain syndromes." Medical
1204 hypotheses **69**(6): 1169-1178.
1205 Pan, Z., W. Zhao, X. Zhang, B. Wang, J. Wang, X. Sun, X. Liu, S. Feng, B. Yang and Y. Lu
1206 (2011). "Scutellarin alleviates interstitial fibrosis and cardiac dysfunction of infarct rats by

inhibiting TGFβ1 expression and activation of p38□MAPK and ERK1/2." British journal of pharmacology **162**(3): 688-700.

Panieri, E., A. Buha, P. Telkoparan-Akillilar, D. Cevik, D. Kouretas, A. Veskoukis, Z. Skaperda, A. Tsatsakis, D. Wallace and S. Suzen (2020). "Potential applications of NRF2 modulators in cancer therapy." Antioxidants **9**(3): 193.

Radan, M., M. Dianat, M. Badavi, S. A. Mard, V. Bayati and M. Ahmadizadeh (2019). "The Association of Cigarette Smoke Exposure with Lung Cellular Toxicity and Oxidative Stress: the Protective Role of Crocin." Inflammation: 1-11.

Rahim, V. B., M. T. Khammar, H. Rakhshandeh, A. Samzadeh-Kermani, A. Hosseini and V. R. Askari (2019). "Crocin protects cardiomyocytes against LPS-Induced inflammation." Pharmacological Reports **71**(6): 1228-1234.

Rahiman, N., M. Akaberi, A. Sahebkar, S. A. Emami and Z. Tayarani-Najaran (2018). "Protective effects of saffron and its active components against oxidative stress and apoptosis in endothelial cells." Microvascular research **118**: 82-89.

Rezaee Khorasany, A., M. Razavi, E. Taghiabadi, A. Tabatabaee Yazdy and H. Hosseinzadeh (2020). "Effect of crocin, an active saffron constituent, on ethanol toxicity in the rat: histopathological and biochemical studies." Iranian Journal of Basic Medical Sciences **23**(1): 51-62.

Rezaee, R., E. Behravan, J. Behravan, F. Soltani, Y. Naderi, B. Emami and M. Iranshahi (2014). "Antigenotoxic activities of the natural dietary coumarins umbelliferone, herniarin and 7-isopentenyloxy coumarin on human lymphocytes exposed to oxidative stress." Drug and chemical toxicology **37**(2): 144-148.

1229 Rezaee, R. and H. Hosseinzadeh (2013). "Safranal: from an aromatic natural product to a
1230 rewarding pharmacological agent." Iranian journal of basic medical sciences **16**(1): 12.
1231 Rezaee, R., K. Jamialahmadi, B. R. Zanjani, M. Mahmoudi, K. Abnous, S. Z. T. Rabe, N.
1232 Tabasi, M. Zali, M. Rezaee and B. Amin (2014). "Crocin effects on human myeloma cells
1233 regarding intracellular redox state, DNA fragmentation, and apoptosis or necrosis profile."
1234 Jundishapur journal of natural pharmaceutical products **9**(4).
1235 Rezaee, R., M. Mahmoudi, K. Abnous, S. Z. T. Rabe, N. Tabasi, M. Hashemzaei and G. Karimi
1236 (2013). "Cytotoxic effects of crocin on MOLT-4 human leukemia cells." Journal of
1237 Complementary and Integrative Medicine **10**(1): 105-112.
1238 Rezaee, R., A. Monemi, M. A. SadeghiBonjar and M. Hashemzaei (2019). "Berberine alleviates
1239 paclitaxel-induced neuropathy." Journal of pharmacopuncture **22**(2): 90.
1240 Rezaei, N., A. Avan, M. Pashirzad, F. Rahmani, R. Moradi Marjaneh, R. Behnam-Rassouli, M.
1241 Shafiee, M. Ryzhikov, M. Hashemzehi and F. Ariakia (2019). "Crocin as a novel therapeutic
1242 agent against colitis." Drug and chemical toxicology: 1-8.
1243 Ribaldone, D. G., R. Pellicano and G. C. Actis (2019). "Inflammation in gastrointestinal
1244 disorders: prevalent socioeconomic factors." Clinical and experimental gastroenterology **12**: 321.
1245 Rong, Y., W. Liu, Z. Zhou, F. Gong, J. Bai, J. Fan, L. Li, Y. Luo, Z. Zhou and W. Cai (2019).
1246 "Harpagide inhibits neuronal apoptosis and promotes axonal regeneration after spinal cord injury
1247 in rats by activating the Wnt/β-catenin signaling pathway." Brain research bulletin **148**: 91-99.
1248 Sadoughi, D. (2019). "The effect of crocin on apoptotic, inflammatory, BDNF, Pt, and Aβ40
1249 indicators and neuronal density of CA1, CA2, and CA3 regions of hippocampus in the model of
1250 Alzheimer suffering rats induced with trimethyltin chloride." Comparative Clinical Pathology
1251 **28**(5): 1403-1413.

Safakhah, H. A., F. Damghanian, A.-R. Bandegi and H. Miladi-Gorji (2020). "Effect of crocin on morphine tolerance and serum BDNF levels in a rat model of neuropathic pain." Pharmacological Reports: 1-9.

Safakhah, H. A., T. Taghavi, A. Rashidy-Pour, A. A. Vafaei, M. Sokhanvar, N. Mohebbi and M. Rezaei-Tavirani (2016). "Effects of saffron (Crocus sativus L.) stigma extract and its active constituent crocin on neuropathic pain responses in a rat model of chronic constriction injury." Iranian journal of pharmaceutical research: IJPR **15**(1): 253.

Saklatvala, J. (2004). "The p38 MAP kinase pathway as a therapeutic target in inflammatory disease." Current opinion in pharmacology **4**(4): 372-377.

Salama, R. M., G. A. Abdel-Latif, S. S. Abbas, M. Hekmat and M. F. Schaalan (2020). "Neuroprotective effect of crocin against rotenone-induced Parkinson's disease in rats: Interplay between PI3K/Akt/mTOR signaling pathway and enhanced expression of miRNA-7 and miRNA-221." Neuropharmacology **164**: 107900.

Samaha, M. M., E. Said and H. A. Salem (2019). "A comparative study of the role of crocin and sitagliptin in attenuation of STZ-induced diabetes mellitus and the associated inflammatory and apoptotic changes in pancreatic β-islets." Environmental Toxicology and Pharmacology **72**: 103238.

Samarghandian, S., M. Azimi-Nezhad and T. Farkhondeh (2016). "Crocin attenuate Tumor Necrosis Factor-alpha (TNF-α) and interleukin-6 (IL-6) in streptozotocin-induced diabetic rat aorta." Cytokine **88**: 20-28.

Samarghandian, S., M. Azimi□Nezhad, A. Borji and T. Farkhondeh (2016). "Effect of crocin on aged rat kidney through inhibition of oxidative stress and proinflammatory state." Phytotherapy research **30**(8): 1345-1353.

1275 Santhosh, M. S., M. S. Sundaram, K. Sunitha, K. Kemparaju and K. Girish (2013). "Viper
1276 venom-induced oxidative stress and activation of inflammatory cytokines: a therapeutic approach
1277 for overlooked issues of snakebite management." Inflammation research **62**(7): 721-731.
1278 Sauter, K. A., L. J. Wood, J. Wong, M. Iordanov and B. E. Magun (2011). "Doxorubicin and
1279 daunorubicin induce processing and release of interleukin-1β through activation of the NLRP3
1280 inflammasome: Progress at a snail's pace." Cancer biology & therapy **11**(12): 1008-1016.
1281 Schuliga, M. (2015). "NF-kappaB signaling in chronic inflammatory airway disease."
1282 Biomolecules **5**(3): 1266-1283.
1283 Shafahi, M., G. Vaezi, H. Shajiee, S. Sharafi and M. Khaksari (2018). "Crocin inhibits apoptosis
1284 and astrogliosis of hippocampus neurons against methamphetamine neurotoxicity via antioxidant
1285 and anti-inflammatory mechanisms." Neurochemical research **43**(12): 2252-2259.
1286 Shahidani, S., Z. Rajaei and H. Alaei (2019). "Pretreatment with crocin along with treadmill
1287 exercise ameliorates motor and memory deficits in hemiparkinsonian rats by anti-inflammatory
1288 and antioxidant mechanisms." Metabolic brain disease **34**(2): 459-468.
1289 Shi, S., Z. Man, W. Li, S. Sun and W. Zhang (2016). "Silencing of Wnt5a prevents
1290 interleukin□1β□induced collagen type II degradation in rat chondrocytes." Experimental and
1291 therapeutic medicine **12**(5): 3161-3166.
1292 Slegtenhorst, B. R., F. J. Dor, H. Rodriguez, F. J. Voskuil and S. G. Tullius (2014).
1293 "Ischemia/reperfusion injury and its consequences on immunity and inflammation." Current
1294 transplantation reports **1**(3): 147-154.
1295 Sommer, C., M. Leinders and N. Üçeyler (2018). "Inflammation in the pathophysiology of
1296 neuropathic pain." Pain **159**(3): 595-602.

1297 Suh, K. S., S. Chon, W.-W. Jung and E. M. Choi (2019). "Crocin attenuates methylglyoxal-
1298 induced osteoclast dysfunction by regulating glyoxalase, oxidative stress, and mitochondrial
1299 function." Food and chemical toxicology **124**: 367-373.
1300 Sung, Y.-Y. and H. K. Kim (2018). "Crocin ameliorates atopic dermatitis symptoms by down
1301 regulation of Th2 response via blocking of NF-κB/STAT6 signaling pathways in mice."
1302 Nutrients **10**(11): 1625.
1303 Takahashi-Yanaga, F. and T. Sasaguri (2008). "GSK-3β regulates cyclin D1 expression: a new
1304 target for chemotherapy." Cellular signalling **20**(4): 581-589.
1305 Tamaddonfard, E., A.-A. Farshid, K. Eghdami, F. Samadi and A. Erfanparast (2013).
1306 "Comparison of the effects of crocin, safranal and diclofenac on local inflammation and
1307 inflammatory pain responses induced by carrageenan in rats." Pharmacological Reports **65**(5):
1308 1272-1280.
1309 Tamaddonfard, E. and N. Hamzeh□Gooshchi (2010). "Effect of crocin on the morphine□
1310 induced antinociception in the formalin test in rats." Phytotherapy Research: An International
1311 Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product
1312 Derivatives **24**(3): 410-413.
1313 Tawfik, S. S. and A. A. Elkady (2019). "Crocin mitigates γ-rays-induced hepatic toxicity in rats."
1314 Environmental Science and Pollution Research **26**(15): 15414-15419.
1315 Terraf, P., S. M. Kouhsari, J. Ai and H. Babaloo (2017). "Tissue-engineered regeneration of
1316 hemisected spinal cord using human endometrial stem cells, poly ε-caprolactone scaffolds, and
1317 crocin as a neuroprotective agent." Molecular neurobiology **54**(7): 5657-5667.

1318 Tsalamandris, S., A. S. Antonopoulos, E. Oikonomou, G.-A. Papamikroulis, G. Vogiatzi, S.
1319 Papaioannou, S. Deftereos and D. Tousoulis (2019). "The role of inflammation in diabetes:
1320 current concepts and future perspectives." European Cardiology Review **14**(1): 50.
1321 Tsantarliotou, M., S. Lavrentiadou, D. Psalla, I. Margaritis, M. Kritsepi, I. Zervos, M. Latsari, V.
1322 Sapanidou, I. Taitzoglou and Z. Sinakos (2019). "Suppression of plasminogen activator
1323 inhibitor-1 (PAI-1) activity by crocin ameliorates lipopolysaccharide-induced thrombosis in
1324 rats." Food and chemical toxicology **125**: 190-197.
1325 Tsatsakis, A., D. Kouretas, M. Tzatzarakis, P. Stivaktakis, K. Tsarouhas, K. Golokhvast, V.
1326 Rakitskii, V. Tutelyan, A. Hernandez and R. Rezaee (2017). "Simulating real-life exposures to
1327 uncover possible risks to human health: a proposed consensus for a novel methodological
1328 approach." Human & experimental toxicology **36**(6): 554-564.
1329 Tsatsakis, A., N. V. Tyshko, A. O. Docea, S. I. Shestakova, Y. S. Sidorova, N. A. Petrov, O.
1330 Zlatian, M. Mach, T. Hartung and V. A. Tutelyan (2019). "The effect of chronic vitamin
1331 deficiency and long term very low dose exposure to 6 pesticides mixture on neurological
1332 outcomes–A real-life risk simulation approach." Toxicology letters **315**: 96-106.
1333 Tsatsakis, A. M., A. O. Docea, D. Calina, A. M. Buga, O. Zlatian, S. Gutnikov, R. N. Kostoff
1334 and M. Aschner (2019). "Hormetic Neurobehavioral effects of low dose toxic chemical mixtures
1335 in real-life risk simulation (RLRS) in rats." Food and chemical toxicology **125**: 141-149.
1336 Tsoukalas, D., V. Fragoulakis, E. Sarandi, A. O. Docea, E. Papakonstantinou, G. Tsilimidos, C.
1337 Anamaterou, P. Fragkiadaki, M. Aschner and A. Tsatsakis (2019). "Targeted metabolomic
1338 analysis of serum fatty acids for the prediction of autoimmune diseases." Frontiers in molecular
1339 biosciences **6**: 120.

1340 Ungurianu, A., D. Margină, D. Grădinaru, C. Băcanu, M. Ilie, C. Tsitsimpikou, K. Tsarouhas, D.
1341 A. Spandidos and A. M. Tsatsakis (2017). "Lipoprotein redox status evaluation as a marker of
1342 cardiovascular disease risk in patients with inflammatory disease." Molecular medicine reports
1343 **15**(1): 256-262.
1344 Van Linthout, S. and C. Tschöpe (2017). "Inflammation–cause or consequence of heart failure or
1345 both?" Current heart failure reports **14**(4): 251-265.
1346 Wang, J.-F., H.-J. Xu, Z.-L. He, Q. Yin and W. Cheng (2020). "Crocin Alleviates Pain
1347 Hyperalgesia in AIA Rats by Inhibiting the Spinal Wnt5a/β-Catenin Signaling Pathway and Glial
1348 Activation." Neural Plasticity **2020**.
1349 Wang, J., Y. Ke and T. Shu (2020). "Crocin has pharmacological effects against the pathological
1350 behavior of colon cancer cells by interacting with the STAT3 signaling pathway." Experimental
1351 and Therapeutic Medicine **19**(2): 1297-1303.
1352 Wang, J., J. Kuai, Z. Luo, W. Wang, L. Wang, C. Ke, X. Li and Y. Ni (2015). "Crocin attenuates
1353 lipopolysacchride-induced acute lung injury in mice." International journal of clinical and
1354 experimental pathology **8**(5): 4844.
1355 Wang, K., L. Zhang, W. Rao, N. Su, H. Hui, L. Wang, C. Peng, Y. Tu, S. Zhang and Z. Fei
1356 (2015). "Neuroprotective effects of crocin against traumatic brain injury in mice: Involvement of
1357 notch signaling pathway." Neuroscience letters **591**: 53-58.
1358 Wang, S.-F., J.-C. Yen, P.-H. Yin, C.-W. Chi and H.-C. Lee (2008). "Involvement of oxidative
1359 stress-activated JNK signaling in the methamphetamine-induced cell death of human SH-SY5Y
1360 cells." Toxicology **246**(2-3): 234-241.

Ware, L. B. (2006). Pathophysiology of acute lung injury and the acute respiratory distress syndrome. Seminars in respiratory and critical care medicine, Copyright© 2006 by Thieme Medical Publishers, Inc., 333 Seventh Avenue, New ….

Wills-Karp, M., J. Luyimbazi, X. Xu, B. Schofield, T. Y. Neben, C. L. Karp and D. D. Donaldson (1998). "Interleukin-13: central mediator of allergic asthma." Science **282**(5397): 2258-2261.

Wu, C.-C., C.-C. Yen, C.-C. Su, F.-C. Tang, K.-L. Chen, Y.-C. Su and Y.-W. Chen (2012). "Involvement of oxidative stress-induced ERK/JNK activation in the Cu2+/pyrrolidine dithiocarbamate complex-triggered mitochondria-regulated apoptosis in pancreatic β-cells." Toxicology letters **208**(3): 275-285.

Wu, T., J. Zhang, M. Geng, S.-J. Tang, W. Zhang and J. Shu (2017). "Nucleoside reverse transcriptase inhibitors (NRTIs) induce proinflammatory cytokines in the CNS via Wnt5a signaling." Scientific reports **7**(1): 1-9.

Xiao, Q., Z. Xiong, C. Yu, J. Zhou, Q. Shen, L. Wang, X. Xie and Z. Fu (2019). "Antidepressant activity of crocin-I is associated with amelioration of neuroinflammation and attenuates oxidative damage induced by corticosterone in mice." Physiology & Behavior **212**: 112699.

Xie, Y., Q. He, H. Chen, Z. Lin, Y. Xu and C. Yang (2019). "Crocin ameliorates chronic obstructive pulmonary disease-induced depression via PI3K/Akt mediated suppression of inflammation." European journal of pharmacology **862**: 172640.

Xiong, Y., J. Wang, H. Yu, X. Zhang and C. Miao (2015). "Anti-asthma potential of crocin and its effect on MAPK signaling pathway in a murine model of allergic airway disease." Immunopharmacology and immunotoxicology **37**(3): 236-243.

1383 Xu, G.-L., G. Li, H.-P. Ma, H. Zhong, F. Liu and G.-Z. Ao (2009). "Preventive effect of crocin
1384 in inflamed animals and in LPS-challenged RAW 264.7 cells." Journal of agricultural and food
1385 chemistry **57**(18): 8325-8330.
1386 Xu, Q. and T. L. Yaksh (2011). "A brief comparison of the pathophysiology of inflammatory
1387 versus neuropathic pain." Current opinion in anaesthesiology **24**(4): 400.
1388 Yalniz, M., U. Demirel, C. Orhan, I. H. Bahcecioglu, I. H. Ozercan, C. Aygun, M. Tuzcu and K.
1389 Sahin (2012). "Nadroparin sodium activates Nrf2/HO-1 pathway in acetic acid-induced colitis in
1390 rats." Inflammation **35**(3): 1213-1221.
1391 Yang, L., Y. Chu, Y. Wang, X. Zhao, W. Xu, P. Zhang, X. Liu, S. Dong, W. He and C. Gao
1392 (2014). "siRNA-mediated silencing of Wnt5a regulates inflammatory responses in
1393 atherosclerosis through the MAPK/NF-κB pathways." International journal of molecular
1394 medicine **34**(4): 1147-1152.
1395 Yang, L. and X. Dong (2017). "Inhibition of inflammatory response by crocin attenuates
1396 hemorrhagic shock-induced organ damages in rats." Journal of Interferon & Cytokine Research
1397 **37**(7): 295-302.
1398 Yang, S. D., J. S. Yu, T. T. Lee, C. C. Yang, M. H. Ni and Y. Y. Yang (1995). "Dysfunction of
1399 protein kinase FA/GSK□3α in lymphocytes of patients with schizophrenic disorder." Journal of
1400 cellular biochemistry **59**(1): 108-116.
1401 Yang, X., F. Huo, B. Liu, J. Liu, T. Chen, J. Li, Z. Zhu and B. Lv (2017). "Crocin inhibits
1402 oxidative stress and pro-inflammatory response of microglial cells associated with diabetic
1403 retinopathy through the activation of PI3K/Akt signaling pathway." Journal of Molecular
1404 Neuroscience **61**(4): 581-589.

Yaribeygi, H., M. T. Mohammadi, R. Rezaee and A. Sahebkar (2018). "Crocin improves renal function by declining Nox□4, IL□18, and p53 expression levels in an experimental model of diabetic nephropathy." Journal of cellular biochemistry **119**(7): 6080-6093.

Yarijani, Z. M., H. Najafi and S. H. Madani (2016). "Protective effect of crocin on gentamicin-induced nephrotoxicity in rats." Iranian journal of basic medical sciences **19**(3): 337.

Yarijani, Z. M., A. Pourmotabbed, T. Pourmotabbed and H. Najafi (2017). "Crocin has anti-inflammatory and protective effects in ischemia-reperfusion induced renal injuries." Iranian journal of basic medical sciences **20**(7): 753.

Yorgun, M. A., K. Rashid, A. Aslanidis, C. Bresgen, K. Dannhausen and T. Langmann (2017). "Crocin, a plant-derived carotenoid, modulates microglial reactivity." Biochemistry and biophysics reports **12**: 245-250.

Yosri, H., W. F. Elkashef, E. Said and N. M. Gameil (2017). "Crocin modulates IL-4/IL-13 signaling and ameliorates experimentally induced allergic airway asthma in a murine model." International immunopharmacology **50**: 305-312.

Yu, X.-J., D.-M. Zhang, L.-L. Jia, J. Qi, X.-A. Song, H. Tan, W. Cui, W. Chen, G.-Q. Zhu and D.-N. Qin (2015). "Inhibition of NF-κB activity in the hypothalamic paraventricular nucleus attenuates hypertension and cardiac hypertrophy by modulating cytokines and attenuating oxidative stress." Toxicology and applied pharmacology **284**(3): 315-322.

Yuan, S., Y. Shi, K. Guo and S.-J. Tang (2018). "Nucleoside reverse transcriptase inhibitors (NRTIs) induce pathological pain through Wnt5a-mediated neuroinflammation in aging mice." Journal of Neuroimmune Pharmacology **13**(2): 230-236.

1426 Zaghloul, M. S., E. Said, G. M. Suddek and H. A. Salem (2019). "Crocin attenuates lung
1427 inflammation and pulmonary vascular dysfunction in a rat model of bleomycin-induced
1428 pulmonary fibrosis." Life sciences **235**: 116794.
1429 Zalar, D.-M., C. Pop, E. Buzdugan, D. Todea and C. I. Mogosan (2019). "THE
1430 ATHEROSCLEROSIS-INFLAMMATION RELATIONSHIP-A PATHOPHYSIOLOGICAL
1431 APPROACH." FARMACIA **67**(6): 941-947.
1432 Zhang, D., B.-y. Qi, W.-w. Zhu, X. Huang and X.-z. Wang (2020). "Crocin alleviates
1433 lipopolysaccharide-induced acute respiratory distress syndrome by protecting against glycocalyx
1434 damage and suppressing inflammatory signaling pathways." Inflammation Research: 1-12.
1435 Zhang, H., S. Wu, Y. Yang, R. Su, J. Wen, X. Ke and W. Chen (2018). "Crocin Protects Human
1436 Umbilical Vein Endothelial Cells from High Glucose-Induced Injury Via Inhibiting the
1437 Endoplasmic Reticulum Stress Response." Current molecular medicine **18**(3): 166-177.
1438 Zhang, L., R. Previn, L. Lu, R.-F. Liao, Y. Jin and R.-K. Wang (2018). "Crocin, a natural
1439 product attenuates lipopolysaccharide-induced anxiety and depressive-like behaviors through
1440 suppressing NF-kB and NLRP3 signaling pathway." Brain research bulletin **142**: 352-359.
1441 Zhang, W. and H. T. Liu (2002). "MAPK signal pathways in the regulation of cell proliferation
1442 in mammalian cells." Cell research **12**(1): 9-18.
1443 Zhu, K., C. Yang, H. Dai, J. Li, W. Liu, Y. Luo, X. Zhang and Q. Wang (2019). "Crocin inhibits
1444 titanium particle-induced inflammation and promotes osteogenesis by regulating macrophage
1445 polarization." International immunopharmacology **76**: 105865.

1446

Highlights:

- Crocin anti-inflammatory function involves suppression of MAPK and NF-kB, but activation of Nrf2.
- Crocin suppresses production/secretion of TNF-α, IL-6, IL-10, IFN-γ and IL-1β (*in vitro* and *in vivo*) and reduces TNF-α and IL-17 in humans.
- Suppression of MAPK and Wnt5α/β-catenin signaling mediates crocin pain killing effects.
- Even though data is limited in humans, the results of preclinical studies regarding anti-inflammatory/anti-nociceptive effects of crocin are promising and warrant further testing in clinical settings.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: