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Review Article

Antioxidant, anti-inflammatory and cytoprotective effects of crocin, a bioactive constituent of saffron, in Alzheimer's and Parkinson's diseases with a focus on molecular mechanisms: A systematic review

Zahra Tayarani-Najaran[1,2], Elham Hadipour[3], Shirin Ramazani[4], Leyli Taghizadeh[5], Elham Ramazani[6,*]

[1]*Medical Toxicology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran*
[2]*Targeted Drug Delivery Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran*
[3]*Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran*
[4]*Healthcare center, Shirvan Health and Treatment Network, North Khorasan University of Medical Sciences, Bojnord, Iran*
[5]*Imam Khomeini Hospital, Shirvan Health and Treatment Network, North Khorasan University of Medical Sciences, Bojnord, Iran*
[6]*Department of Biology, Yazd University, Yazd, Iran*

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**** Corresponding Author:***
Tel: +98 03531232216
Fax: +98 03538211279
el.ramazani@yazd.ac.ir

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Abstract

Objective: This study assessed the neuroprotective effects of crocin one of the main bioactive compounds of saffron, in Alzheimer's and Parkinson's diseases, by focusing on its anti-oxidative, anti-inflammatory, and cytoprotective properties.

Materials and Methods: In this systematic review, we evaluated the efficacy of crocin on *in vivo* models of Alzheimer's and Parkinson's diseases. Using three online literature databases (PubMed, Scopus, and Google Scholar), we identified studies describing the neuroprotective effects of crocin in Alzheimer's and Parkinson's diseases. A literature search was carried out using a combination of keywords such as crocin, Alzheimer's disease, Parkinson's disease, antioxidant, anti-inflammatory, and cytoprotective. Papers were identified to describe the neuroprotective effects of crocin from 2013 until 2024.

Results: The total number of articles included in the present review is 28. Reducing reactive oxygen species (ROS) and malondialdehyde (MDA) and increasing superoxide dismutase (SOD) levels indicate the anti-oxidant effects of crocin. Crocin can show anti-inflammatory activities via decreasing tumor necrosis factor-alpha (TNF-α) and interleukin levels. Crocin can display cytoprotective effects via down-regulation of p- extracellular signal-regulated kinase (ERK)1/2, and augmentation of phosphoinositide 3-kinases (PI3K)/Akt/ mammalian target of rapamycin (mTOR) signaling pathway activity.

Conclusion: Finally due to a noticeable efficacy of crocin, it is suggested that crocin can be used as a suitable neuroprotective agent against Alzheimer's and Parkinson's diseases.

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Introduction

Neurodegenerative diseases are referred to as the inexorable loss of neurons in the nervous system, which cause a wide range of neurological disorders including Alzheimer's and Parkinson's diseases (Chi et al. 2018; Dugger and Dickson 2017; Hong 2009). One of the important neuropathological hallmarks of neurodegenerative diseases is aberrant protein aggregates which cause apoptotic and non-apoptotic neuronal cell death by various mechanisms (Chi et al. 2018). Increasing evidence detected that natural products and their active components may provide neuroprotective potential to prevent and ameliorate neurodegenerative diseases (Di Paolo et al. 2019).

Alzheimer's disease (AD)

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases and the main leading cause of dementia. Alzheimer's disease is mostly related to significant memory impairment and cognitive deficit, which affects people aged 65 and older (Scheltens et al. 2021; Yu et al. 2021). The symptoms of the disease typically progress from the mildest stage to the most severe stage, which results in neuronal death and neuropathologic lesions in many brain regions (Mantzavinos and Alexiou 2017). Based on the neuropathological studies, some of the biomarkers related to AD pathology are the accumulation of extracellular amyloid beta (Aβ) plaques and intracellular neurofibrillary tangles (NFTs) composed of phosphorylated tau protein (Graff-Radford et al. 2021; Zetterberg and Burnham 2019). Therefore, AD has been decided as a multifactorial and heterogeneous disease associated with important risk factors including advanced age, genetic factors, environmental and metabolic risk factors, infectious, immune system dysfunction, and head injuries (Breijyeh and Karaman 2020; Iqbal and Grundke-Iqbal 2010; Sheppard and Coleman 2020) Today, acetylcholinesterase inhibitors (AChEIs) or antagonists of N-methyl-D-aspartate receptor (NMDAR) as current AD treatments are approved to reduce cognitive symptoms with modest efficacy (Glynn-Servedio and Ranola 2017). Despite numerous studies, effective therapeutic drugs for AD to prevent or stop the rate of disease progression, are not clear (Yu et al. 2021). Although based on new findings, flavonoids, gingerols, tannins, anthocyanins, triterpenes, and alkaloids are considered natural compounds with anti-oxidant and anti-inflammatory activities and may be capable of diminishing the symptoms and progression of several diseases including AD (Noori et al. 2021).

Parkinson's disease (PD)

Parkinson's disease (PD) is the most common neurodegenerative disorder after AD in the world (Beitz 2014). Parkinson's disease is determined by a wide range of motor (rest tremor, rigidity, bradykinesia, gait alterations, freezing of gait, and imbalance) and non-motor symptoms and complications (disorders of sleep, neuropsychiatric and autonomic symptoms, pain, other somatosensory disturbances, cognitive decline and dementia), which can affect function and quality of life 1% of the population above 60 years (Bloem et al. 2021; Jankovic 2008; Tolosa et al. 2021; Tysnes and Storstein 2017). The main neuropathological hallmarks related to PD are nigrostriatal dopaminergic neuron degeneration and intracytoplasmic α-synuclein-containing Lewy bodies in the pars compacta of the substantia nigra (SN) (Geut et al. 2020). PD is a multifactorial, very heterogeneous, and extremely complex neurodegenerative disorder deriving from genetic mutation, aging, and environmental toxin factors (Hayes 2019; Pang et al. 2019). Genetic factors contribute to 3-5% of PD in most populations, and genetic subtypes and

common genetic variants are strongly related to increased risk of PD (Bloem et al. 2021; Tolosa et al. 2021). Recent trends in PD scientific research have focused on identifying therapies that slow or suppress PD progression (Ntetsika et al. 2021). Besides levodopa as a primary treatment for PD, there are many new technological approaches such as gene manipulation methods, deep brain stimulation (DBS) of the subthalamic nucleus procedure, and neuroimaging, that can be more effective in detecting disease signatures and treating PD-related motor symptoms (Ntetsika et al. 2021; Rao et al. 2006). Given the high prevalence and adverse health impacts of non-motor symptoms and despite numerous studies, there is still a need to find the most appropriate treatment (Seppi et al. 2019). A limited number of herbal extracts such as *Hibiscus asper* Hook. f., *Ginkgo biloba* L., *Carthamus tinctorius* L., *Cinnamomum verum*, *Cinnamomum cassia* and *Sesamum indicum* L. and their active compounds such as berberine, curcumin, cinnamaldehyde, sesamin, sesamolin and 6-gingerol have shown anti-oxidant, anti-inflammatory and cytoprotective effects in different animal models of PD (Rabiei et al. 2019; Ramazani et al. 2023; Ramazani et al. 2020; Rezazadeh-Shojaee et al. 2022).

Crocin

Crocus sativus L., saffron, belongs to the family Iridaceae with wide distribution over the world, for example, France, Greece, Italy, Spain, Azerbaijan, Turkey, India, Iran, China, Morocco, Egypt, and Mexico (Alavizadeh and Hosseinzadeh 2014; Bastani et al. 2022; Hosseinzadeh and Nassiri-Asl 2013). Saffron, the dried stigma of the *C. sativus* flower has long been considered one of the most popular medicinal herbs, and it is extensively used as a spice also as a colorant agent both for food and cosmetic products worldwide (Alavizadeh and Hosseinzadeh 2014; Bastani et al. 2022; Javadi et al. 2013; Razavi and Hosseinzadeh 2015). Saffron contains several non-volatile compounds of which about 30% are crocins (Bathaie et al. 2014; Nassiri-Asl and Hosseinzadeh 2015). The crocins, a group of water-soluble carotenoids, comprise a group of mono-or di-glycosyl polyene esters of crocetin (8, 8′-diapocarotene-8,8′-dioic acid) and extracted from saffron (*Crocus sativus* L.) and *Gardenia jasminoides* (Alavizadeh and Hosseinzadeh 2014; Bastani et al. 2022; Nassiri-Asl and Hosseinzadeh 2015). Crocin (syn. α-crocin or crocin 1) is the main saffron-coloring pigment with the scientific name of trans-crocetin di-(β-D-gentiobiosyl) ester, the chemical formula $C_{44}H_{64}O_{24}$, and the molar mass 976.972 g/mol (Ali et al. 2022; IA et al. 2021). Several studies considered the beneficial biological and pharmacological effects of crocin on many organs such as the cardiovascular, gastrointestinal, endocrine, immune, genital, especially nervous system, *etc.* (Alavizadeh and Hosseinzadeh 2014; Boozari and Hosseinzadeh 2022; José Bagur et al. 2017). Its pharmacological activities in the treatment of atherosclerosis, hyperlipidemia, hypertension, myocardial injury, renal failure, hepatic damage, ischemia, antidote, genotoxicity, miscellaneous, cancer, COVID-19, diabetes, cerebral ischemia, depression and anxiety, convulsion, via reducing inflammation, stress oxidative based on recent findings have also been revealed (Alavizadeh and Hosseinzadeh 2014; Bastani et al. 2022). Remarkably, recent studies conveyed that crocin can inhibit inflammatory, apoptotic, and antioxidant processes via various signaling pathways which led to consider this compound as a safe and effective promising option for the treatment of neurodegenerative illnesses, especially Parkinson's disease (Ahmed et al. 2020; Goyal et al. 2023). Furthermore, in comprehensive review study reported that crocin a as medicinal plant showed numerous therapeutic effects in autoimmune diseases such as asthma,

rheumatoid arthritis, type 1 diabetes, ulcerative colitis, and multiple sclerosis (Korani et al. 2019). Moreover, Colapietro et al. reported that saffron and crocin showed chemopreventive properties via antioxidant effects, cancer cells apoptosis, cell proliferation inhibition, augmentation of cell differentiation, cell growth and metabolism, motivation of cell-to-cell communication and immune modulation (Colapietro et al. 2019). Additionally, a comprehensive study by Shafiee and colleagues reported that saffron and its active constituents especially crocin exhibited antidepressant effects similar to current antidepressant medications including fluoxetine, imipramine and citalopram, with fewer side effects (Shafiee et al. 2018). In addition, in a study, Rezaei et al. presented crocin as a new therapeutic agent with low toxicity against colitis. They reported that crocin provoked anti-inflammatory responses and weakened body weight loss, rectal bleeding, diarrhea, and colon shortening in dextran sodium sulfate (DSS)-induced colitis mice (Rezaei et al. 2020). It is worth mentioning that crocin at pharmacological doses showed high efficacy and did not cause major cytotoxic effects or cell damage in experimental models (Alavizadeh and Hosseinzadeh 2014). Due to the numerous pharmacological properties of saffron, as well as the anti-inflammatory, antioxidant, and protective effects of crocin against cancers such as colon cancer, brain injury, cardiac dysfunction, cardiopulmonary dysfunction, respiratory distress, and renal damages, most of the pharmacological and therapeutic studies are focused to these compounds (Abdulkareem Aljumaily et al. 2021; Bakshi et al. 2022; Hosseini et al. 2018; Salem et al. 2022; Zhang et al. 2020).

In this systematic review, we aimed to provide a comprehensive overview of the neuroprotective effects of crocin in Alzheimer's and Parkinson's diseases based on the anti-oxidative, anti-inflammatory, and cytoprotective properties. In addition, we provide a deeper insight into the molecular mechanisms of crocin. Due to few studies of these beneficial effects in humans, also the limitation of confirmation of these effects in humans through clinical trials, as well as accomplished *in vitro* tests with non-physiological metabolites and/or concentrations, in this study the anti-inflammatory, antioxidant, and cytoprotective effects of crocin on *in vivo* models have been considered to achieve in-depth insights of crocin effects on AD and PD.

Materials and Methods

Search Strategy

Based on the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines (Page et al. 2021), we conducted a comprehensive literature search in English using the electronic databases of PubMed, Scopus, and Google Scholar from 2013 until 2024. Based on the numerous studies on saffron and its bioactive constituent crocin in various neurodegenerative disorders all over the world especially in Iran, the time, language and database restrictions were applied to the search strategy. Studies limited to *in vivo* studies in English and non-comparative studies, systematic reviews and meta-analyses, *in vitro* studies, and opinion and editorials excluded. The combination of keywords used for the literature research was as follows: ("crocin") AND ("Alzheimer's disease" OR "Parkinson's disease") OR other related words including ("anti-oxidant" OR "anti-inflammatory" OR "cytoprotective" OR "neuroprotective" OR "anti-apoptotic").

Study selection/ Inclusion and exclusion criteria

To evaluate the neuroprotective potential of crocin, we performed a careful literature search in scientific databases to

find publications that evaluated the mechanisms of action of crocin on *in vivo* models of Alzheimer's and Parkinson's diseases. A selection of relevant references was initially screened based on the title and abstract, and then on the full text of the paper.

Specific inclusion criteria for *in vivo* studies included the following: (1) published in English; (2) administered crocin alone; (3) experimental AD or PD was induced in rodents (i.e., rats or mice); and (4) published in peer-reviewed journals. Additionally, irrelevant documents, reviews or letters, editorials, incomplete articles, duplicates, book chapters, conference papers and experiments without testing the efficacy of crocin on the *in vivo* model of AD or PD were excluded.

Data extraction

Two individual authors (Elham Ramazani and Zahra Tayarani-Najaran) performed separate data collection, screened papers, extracted and classified their information based on (1) the first author's name, publication year and experimental models, (2) individual data were acquired including species, sex and number from each animal and AD or PD models induced agent, (3) and finally, duration of treatment and dosage, route of administration, methods and outcome measures selected.

Risk of bias in individual studies

The checklist of the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) based on the Cochrane Collaboration RoB Tool was used to assess the risk of bias in individual studies (Hooijmans et al. 2014).

Results

Results of the search

Based on our comprehensive search of the electronic databases from 2013 to 2024, we identified a total of 358 references (crocin on AD (n = 204), and crocin on PD (n = 154)). After removing duplicates, the first screening yielded 284 articles: crocin on AD (n = 158), and crocin on PD (n = 126). In the second screening based on the titles and abstracts, we discarded 79 papers with at least one of the subsequent reasons: (1) irrelevant documents, reviews or letters, incomplete articles, and conference papers. Finally, after using full-text criteria, 28 studies were detected to be eligible for the current review and evaluated in this investigation: crocin on AD (n = 18), and crocin on PD (n = 10). In Figure 1, the search strategy is summarized using a PRISMA flowchart (Table 1).

Results of the risk of bias in individual studies

It was recognized that 21 studies had a low risk of bias and three studies had a medium risk (Table 2).

Table 1. Overview of the PICO eligibility criteria

Participants	Animal models of AD and PD subjected to crocin There will be no restrictionon type of animal model and doses of crocin
Intervention	Crocin that has Antioxidant, anti-inflammatory and cytoprotective effects on AD and PD animal models
Comparator	Comparison will be between control and experiment intervention. Studies without a control group will be excluded.
Outcomes	Our primary outcome is inhibition in stress oxidative, inflammation and apoptosis. Studies without inhibitory effects on AD and PD will be excluded. Our secondary outcome is a significant depletion of factors involved in antioxidant, inflammatory, and apoptotic processes.
Study design	Studies will be limited to *in vivo* studies in English using the electronic databases of PubMed, Scopus, and Google Scholar from 2013 until 2024. Non-comparative studies, systematic reviews and meta-analyses, *in vitro* studies, and opinion and editorials will be excluded.

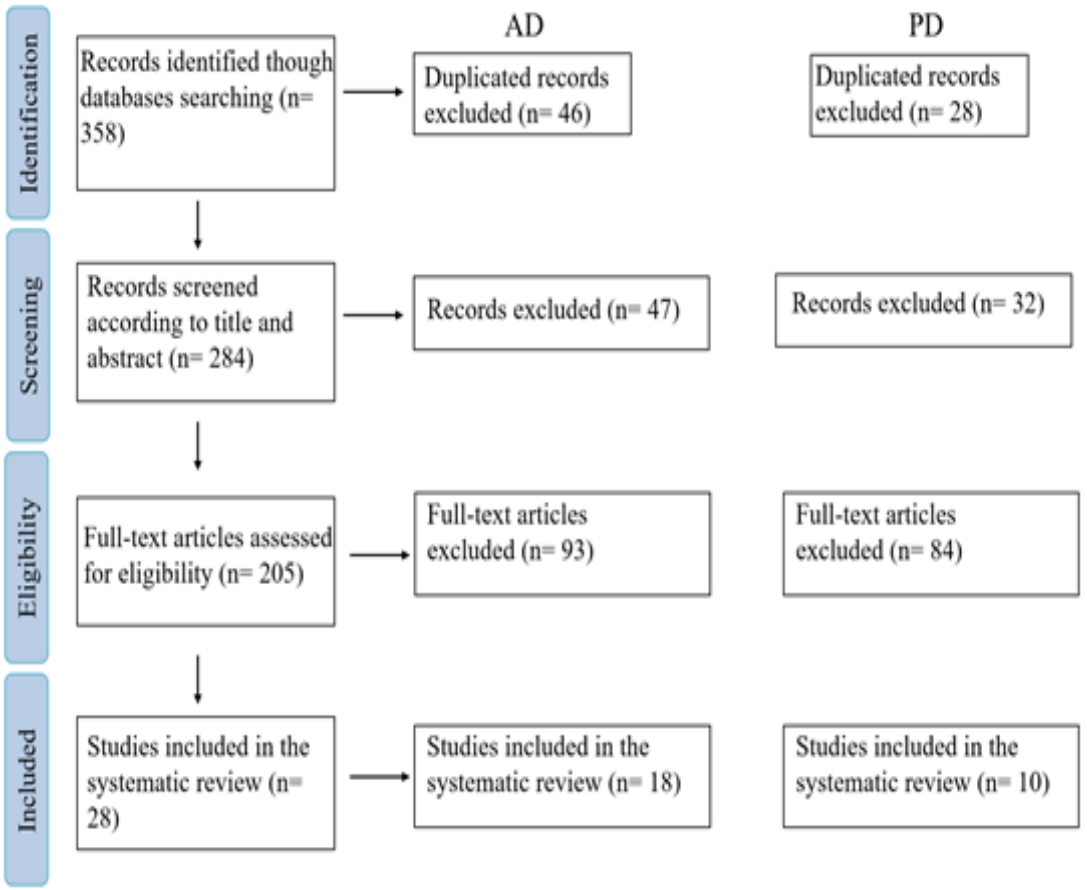

Figure 1. Research methodology for review process according to PRISMA flowchart.

Table 2. Risk of Bias in included studies.

study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Risk
Wang et al. 2019	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Yousefsani et al. 2021	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Mohammadzadeh et al. 2020	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Medium
Naghizadeh et al. 2013	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Naghizadeh et al. 2014	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Rashedinia et al. 2015	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Abbaszade-Cheragheali et al. 2023	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Dastan 2024	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Hadipour et al. 2021	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Sadoughi 2019	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Su 2024	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Moghadasi 2024	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Hadipour et al. 2021	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Hadipour et al. 2018	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Lin et al. 2019	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Asadi et al. 2015	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Ghotbeddin et al. 2021	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Medium
Truski et al. 2020	Yes	Unclear	Unclear	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Medium

Table 2. Continue

Mohammadzadeh et al. 2018	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Hosseini et al. 2016	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Rajaei et al. 2016	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Shahidani et al. 2019	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Abeer and Ahmed 2019	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Salama et al. 2020	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Haeri et al. 2019	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Tang et al. 2020	Yes	Yes	Yes	Unclear	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Alizadehmoghaddam 2024	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low
Mohammadzadeh et al. 2022	Yes	Yes	Yes	Yes	Unclear	Unclear	Unclear	Yes	Yes	Yes	Low

Note: Q1: Was the allocation sequence adequately generated and applied? Q2: Were the groups similar at baseline or were they adjusted for confounders in the analysis? Q3: Was the allocation adequately concealed? Q4: Were the animals randomly housed during the experiment? Q5: Were the caregivers and investigators blinded to the intervention that each animal received? Q6: Were animals selected at random for outcome assessment? Q7: Was the outcome assessor blinded? Q8: Were incomplete outcome data adequately addressed? Q9: Are reports of the study free of selective outcome reporting? Q10: Was the study free of other problems that could result in a high risk of bias?

Study characteristics

The information of articles, including the name of the first author and the year of publication, species, sex and the number of animal models (rodents i.e. rats or mice), AD and PD induced agent (dosage), route of administration and duration, crocin dosage, route of administration and duration, research methods were extracted and summarized in Table 3.

Neuroprotective mechanisms of crocin

Table 3 displays the main outcome measures and findings of the included studies. Twenty-five studies assessed the neuroprotective effects of crocin in AD and PD; crocin on AD (n = 15), and crocin on PD (n = 9). Figure 2 shows the neuroprotective mechanism of crocin in AD.

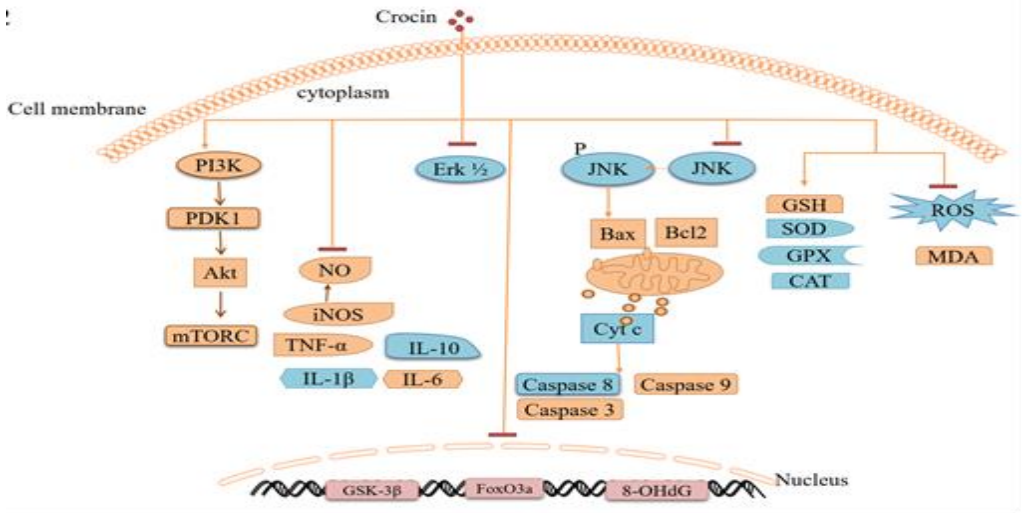

Figure 2. Intracellular signaling pathway through antioxidant, anti-inflammatory, and cytoprotective effects of crocin in AD (blue) and PD (purple) and both of them (orange). Crocin can exert its neuroprotective properties in AD and PD by reducing ROS, MDA and various inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10) and enzymes (iNOS) level, improving the PI3k/Akt/mTOR pathways, increasing bcl-2, GSH, SOD and CAT level, down-regulating of Erk1/2, JNK, pathways and decreasing bax, caspase 3 and 9 level. AD, Alzheimer's disease; Akt, Protein kinase B; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; CAT, Catalase; ERK1/2, Extracellular signal-regulated kinase 1/2; FoxO3a, Forkhead box transcription factor of the O class; GPx, Glutathione peroxidase; GSH, Glutathione; GSK-3β, Glycogen synthase kinase-3β; IL, interleukin; iNOS, Inducible nitric oxide synthase; JNK, c-Jun N-terminal kinase; mTOR, Mammalian target of rapamycin; NO, Nitric oxide; 8-OHdG, 8-Hydroxydeoxyguanosine; PD, Parkinson's disease; PI3K Phosphoinositide 3-kinases; ROS, Reactive oxygen species; SOD, Superoxide dismutase; TNF-α, Tumour necrosis factor-alpha.

Table 3. Basic information of included studies and *in vivo* neuroprotective mechanisms of crocin in AD and PD.

First author (Year)	Animal Models	Administration (dosage, time and route)	Research methods	Outcome measures	Neuroprotection mechanism
Crocin in AD					
Wang (2019)	BALB/c Mice (male, 60) D-galactose (120 mg/kg; 8 weeks; i.p.) and aluminum trichloride (20 mg/kg, 8 weeks; i.g.)	5 or 20 mg/kg (28 days, from the 5th week; i.g.)	Biochemical detection (BCA assay and enzyme-linked immunosorbent assay) Histological examination (hematoxylin-eosin /H&E) Immunohistochemistry	↓ The $A\beta_{1-42}$ content in the cerebral cortex. ↑ The levels of SOD, GPx, choline acetyltransferase, and acetylcholine. ↓ ROS and acetylcholinesterase levels in the serum, cerebral cortex and hypothalamus.	Anti-oxidant
Yousefsani(2021)	Wistar Rat (male, 42) $A\beta_{1-42}$ (0.1 µg/µL, 0.5 µL per side; 21 days; i.h.)	30 mg/kg (seven days with/before/after $A\beta_{1-42}$ administration, i.p.)	Biochemical detection (mitochondrial tests)	↓ The amount of mitochondrial ROS formation, mitochondrial lipid peroxidation. ↑ GSH level. ↓ Cytochrome c expulsion from mitochondria and mitochondrial outer membrane damages. ↑ MMP.	Anti-oxidant and anti-apoptotic
Mohammadzadeh (2020)	Wistar Rat (male, 36) Malathion (100 mg/kg; 14 days; i.p.)	10, 20 and 40 mg/kg (14 days, i.p.)	Biochemical detection (cholinesterase activity assay, oxidative stress and inflammatory markers) Western Blot RT-PCR	↓ MDA level. ↑ GSH level. ↓ TNF-α and IL-6 level. ↓ Bax/Bcl2 ratio, cleaved caspase-3, 8 and 9 protein level, and Tau mRNA expression.	Anti-oxidant, anti-inflammatory and anti-apoptotic
Naghizadeh (2013, 2014)	Wistar Albino Rat (male, 20) Streptozotocin (STZ) (3 mg/kg; bilaterally, on days 1 and 3; i.c.v.)	100 mg/kg (21 days; i.c.v.)	Biochemical detection (thiobarbituric acid reactive substance assay (TBARS) assay, Total thiol (-SH) groups assay, GSH peroxidase concentration)	↓ MDA level. ↑ Total thiol content and GPx activity.	Anti-oxidant
Rashedinia (2015)	Wistar Rat (male, 36) Acrolein (3 mg/kg; 2 weeks; i.p.)	12.5, 25 and 50 mg/kg (2 weeks; i.p.).	Biochemical detection (lipid peroxide levels, glutathione (GSH) levels, and Aβ levels) Western Blot	↓ MDA, $A\beta_{1-42}$ and phospho-tau level via down-regulating p-Akt, p-ERK1/2, and p-SAPK/JNK.	Anti-oxidant
Abbaszade-Cheragheali (2023)	Wistar Rat (male) Unpredictable chronic mild stress (UCMS) (4 weeks)	10, 20, and 30 mg/kg (4 weeks)	Biochemical detection (oxidative stress and inflammatory markers)	↓ MDA and nitrite level. ↑ Total thiol content, SOD, and CAT activity. ↓ TNF-α and IL-10 level. ↓ Aβ level.	Anti-oxidant, anti-inflammatory and anti-apoptotic
Dastan (2024)	Wistar Rat (male) Lipopolysaccharides (LPS) (1 mg/kg; 5 days; i.p.)	100 mg/kg (12 days; i.p.)	Real-time PCR	↓ The mRNA expression of NF-κB, TNF-α, caspase 3 and lipid peroxidation in the hippocampus.	Anti-oxidant, anti-inflammatory and anti-apoptotic
Hadipour (2021)	Wistar Rat (male, 40) $A\beta_{1-42}$ (12 days; i.p.)	30 mg/kg (12 days; i.p.)	Biochemical detection (inflammatory markers)	↓ TNF-αand IL-1β mRNA level.	Anti-inflammatory

Table 3. Continue

Sadoughi (2019)	Wistar Rat (male, 56) Trimethyltin chloride (TMT) (8 mg/kg; i.p.)	25 and 50 mg/kg (i.p.)	Biochemical detection (inflammatory markers) Western Blot	↓ TNF-α, IL-1β, and IL-6 level. ↑ Bcl-2, BDNF, and neuronal density of CA1, CA2, and CA3 regions. ↓ Bax, caspase-9, Pt, and $A\beta_{40}$ levels in the rat hippocampus.	Anti-inflammatory and anti-apoptotic
Su (2024)	ICR Mice (male, 30) $A\beta_{25\text{-}35}$ (5 μg/μl; injected into the hippocampus of mice, specifically, the anteroposterior (AP) was 2.0 mm, the mediolateral (ML) was 2.0 mm, and the dorsoventral (DV) was 1.7 mm)	40 mg/kg (i.p.; 14 days)	Histological examination (hematoxylin-eosin /H&E) Western Blot qRT-PCR	↓ IL-1β, IL-6, and TNF-α	Anti-inflammatory
Moghadasi (2024)	Sprague Dawley Rat (male, 40) TMT (8 mg/kg; i.p.)	25 mg/kg (i.p.; 8 weeks)	qRT-PCR	↑ NGF, BDNF, and TrkB gene expression ↓ Tau gene expression	anti-apoptotic
Hadipour (2021)	Wistar Rat (male, 40) $A\beta_{1\text{-}42}$ (12 days; i.p.)	30 mg/kg (12 days; i.p.)	Cresyl violet, Golgi-Cox and TUNEL staining	↑ Number of live cells. ↓ Apoptotic cell number. Ameliorated the spine, axonal, and dendrites arborization in the CA3 region and frontal cortex of the rat.	Anti-apoptotic
Hadipour (2018)	Wistar Rat (male, 40) $A\beta_{1\text{-}42}$ (12 days; i.p.)	30 mg/kg (12 days; i.p.)	TUNEL assay Western Blot	↓ The number of TUNEL- positive cells in the CA1 region and c-Fos in the rat hippocampus.	Anti-apoptotic
Lin (2019)	Wistar Rat (male, 48) $A\beta_{25\text{–}35}$ ((5 μL; 14 days; i.c.v.)	40 mg/kg (14 days; i.p.)	TUNEL Assay Immunofluorescent Staining Western Blot	↓ Apoptotic cell number. ↑ Bcl-2 expression level. ↓ Bax, Caspase3, GRP78, and CHOP expression levels.	Anti-apoptotic
Asadi (2015)	Wistar Rat (male,) $A\beta_{1\text{-}42}$ (50 ng/side; i.h.)	150, 300 and 600 nmol (i.p.)	Western Blot	↓ Bax/Bcl-2 ratio and cleaved caspase-3 level.	Anti-apoptotic
Ghotbeddin (2021)	Wistar Rat (female,42) Hypoxia (under 7% O2 + 93% N2 condition for 3 hr; on the 20th day of pregnancy)	30 mg/kg (from P14 to P27; i.p.)	RT-PCR	↓ Production of β-amyloid (βA_{42} and βA_{40}) of APP of the neonate rat brain on the gestational hypoxia through decreasing HIF-1 α and BACE1 mRNA level.	Anti-apoptotic
Truski (2020)	Wistar Rat (female,40) Hypoxia (under 7% O2 + 93% N2 condition for 3 hr; at p14)	30 mg/kg (at p14 (received hypoxia); i.p.)	qRT-PCR	↓ β-amyloid production following down-regulation of HIF-1α and BACE1 mRNA level.	Anti-apoptotic
Crocin in PD					
Mohammadzadeh (2018)	Wistar Rat (male) Malathion (100 mg/kg; 28 days; i.p.)	10, 20, and 40 mg/kg (28 days; i.p.)	Biochemical detection (oxidative stress and inflammatory markers)	↓ MDA level. ↑ GSH level. ↓ TNF-α and IL-6 levels.	Anti-oxidant, anti-inflammatory
Hosseini (2016)	Wistar Rat (male, 32) 6-OHDA (16μg in 0.2% ascorbate-saline; 6 weeks; microinjection into the left medial forebrain (MFB))	30 and 60 mg/kg (6 weeks; i.p.)	Biochemical detection (lipid peroxide levels, glutathione peroxidase (GPx) levels, and nitrite levels)	↓ Nitrite level.	Anti-oxidant

Table 3. Continue

Rajaei (2016)	Wistar Rat (male, 32) 6-OHDA (16µg in 0.2% ascorbate-saline; 6 weeks; microinjection into the left medial forebrain (MFB))	30 and 60 mg/kg (6 weeks; i.p.)	Biochemical detection (lipid peroxide levels, glutathione peroxidase (GPx) levels, and nitrite levels)	↓ TBARS and nitrite level.	Anti-oxidant
Shahidani (2019)	Wistar Rat (male, 37) 6-OHDA (16µg in 0.2% ascorbate-saline; 6 weeks; microinjection into the left medial forebrain (MFB))	100 mg/kg (6 weeks; i.p.)	Biochemical detection (lipid peroxide levels, cytokine levels, and Total thiol concentration)	↓ Lipid peroxidation level. ↓ TNF-α level.	Anti-oxidant and anti-inflammatory
Abeer (2019)	Albino Rat (male, 70) ROT (i.p.)	20 and 40 mg/Kg (4 weeks; i.p.)	Biochemical detection (oxidative stress and inflammatory markers)	↓ MDA, 8-OHdG, and nitrite/nitrate levels. ↑ GSH level. ↓ TNF-α level.	Anti-oxidant and anti-inflammatory
Salama (2020)	Wistar Rat (male, 60) ROT (1.5 mg/kg; 30 days; i.p.)	30 mg/kg (30 days; i.p.)	Immunohistochemistry RT-PCR Western Blot	↑ TH level. ↑ PI3K/Akt/mTOR signaling pathway activity. ↑ miRNA-7 and miRNA-221 expression levels as activators of Akt/mTOR. ↓ GSK-3β, FoxO3a, caspase-9, and α-synuclein level. ↑ Dopamine level.	Anti-oxidant and anti-apoptotic
Haeri (2019)	BALB/c Mice (male, 24) MPTP (30 mg/kg for 5 days; i.p.)	30 mg/kg (15 days; i.p.)	Immunohistochemistry Toluidine blue assay	↑ The number of TH-positive neurons. ↑ The number of dark neurons. ↓ Cell death in the substantia nigra.	Anti-oxidant and anti-apoptotic
Tang (2020)	C57BL/6 Mice (male, 60) MPTP (30 mg/kg; 7 days; i.p.)	(for 7 days after MPTP administration; i.g.)	Biochemical detection (tyrosine Hydroxylase (TH) immunostaining Western Blot	↑ The expression of TH in VTA and mPFC. ↓ The PDD symptoms in the VTA through improving mTOR signaling.	Anti-oxidant and anti-apoptotic
Alizadehmoghaddam 2024	Wistar Rat (male, 50) LPS (1 µg/µl; 3 weeks; i.p.)	15 and 30 mg/kg (before and after LPS; 4 weeks, i.p.)	Biochemical detection (immunofluorescence staining for inflammatory markers and TH immunoblotting) qRT-PCR	↓ Number of caspase-1 and IL-1β positive cells, IL-1β, IL-18, NLRP1, and AIM2 genes expression ↑ TH expression	Anti-inflammatory
Mohammadzadeh (2022)	Wistar Rat (male) Malathion (100 mg/kg; 28 days; i.p.)	10 and 40 mg/kg (28 days; i.p.)	Western Blot RT-qPCR	↓ Bax/Bcl2 ratio, caspases-3, 9 protein levels, and mRNA level of α-synuclein.	Anti-apoptotic

Abbreviations: Aβ, Amyloid beta; AD, Alzheimer's disease; Akt, Protein kinase B; APP, Amyloid precursor protein; BACE1, β-secretase; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; BDNF, Brain-derived neurotrophic factor; CAT, Catalase; CHOP, C/EBP homologous protein; DG, Dentate gyrus; ERK1/2, Extracellular signal-regulated kinase 1/2; FoxO3a, Forkhead box transcription factor of the O class; GPx, Glutathione peroxidase; GRP78, Glucose regulated protein 78; GSH, Glutathione; GSK-3β, Glycogen synthase kinase-3β; HIF-1, Hypoxia-inducible factor-1; ICV, Intracerebroventricular; IH, Intra-hippocampally; IL, interleukin; iNOS, Inducible nitric oxide synthase; IP, Intraperitoneal; JNK, c-Jun N-terminal kinase; LPS, Lipopolysaccharide; MDA, Malondialdehyde; MMP, Mitochondrial membrane potential; mPFC, Medial prefrontal cortex; MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mTOR, Mammalian target of rapamycin; NACE, Non-aqueous capillary electrophoresis; NFTs, Neurofibrillary tangles; NGF, Nerve growth factor; NMDAR, N-methyl-D-aspartate receptor; NO, Nitric oxide; 6-OHDA, 6-Hydroxydopamine; 8-OHdG, 8-Hydroxydeoxyguanosine; PD, Parkinson's disease; PI3K Phosphoinositide 3-kinases; Pt, Phosphor tau protein; RNS, Reactive nitrogen species; ROS, Reactive oxygen species; ROT, Rotenone; SAPK, Stress-activated protein kinase; SN, Substantia nigra; SOD, Superoxide dismutase; STZ, Streptozotocin; TBARS, Thiobarbituric acid reactive substances; TH, Tyrosine hydroxylase; TrkB, Tyrosine kinase B; TMT, Trimethyltin chloride; TNF-α, Tumour necrosis factor alpha; TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling; UCMS, Unpredictable chronic mild stress; VTA, Ventral tegmental area.

Discussion

In vivo neuroprotective mechanisms of crocin in AD

Several investigations have shown oxidative stress and inflammatory responses exhibited critical roles in various neurodegenerative diseases, especially AD. Because of the potential health benefits of crocin in AD, numerous studies have been conducted in this field. Among all the pharmacological properties of crocin, there is more evidence reporting the anti-oxidant, anti-inflammatory and cytoprotective effects of crocin, and we summarized those studies which include the rat/mice model of AD. The results of these *in vivo* studies on neuroprotective properties of crocin in AD are detailed in Table 3 & summarized in Figure 2.

Anti-oxidant properties of crocin in AD

The anti-oxidant activities of crocin in AD were assessed in six studies. Wang et al. investigated the anti-oxidant properties of crocin in d-galactose (120 mg/kg/day) and aluminium trichloride (20 mg/kg/day)-induced AD in mice for 8 weeks. They reported that intragastric administration of crocin (5 or 20 mg/kg; 28 days; from the 5th week) significantly increased the amyloid β-peptides $(A\beta)_{1-42}$ content in the serum, and decreased the $A\beta_{1-42}$ content in the cerebral cortex using an enzyme-linked immunosorbent assay AD mice. After crocin therapy, the levels of glutathione peroxidase (GPx), superoxide dismutase (SOD), choline acetyltransferase, and acetylcholine also augmented. Inhibition of reactive oxygen species (ROS) and acetylcholinesterase levels in the serum, cerebral cortex and hypothalamus is another mechanism by which crocin can exert its anti-oxidant effects in AD mice (Wang et al. 2019). Additionally, Yousefsani et al. reported that crocin has neuroprotective effects in rat models of AD. Results indicated that pretreatment with crocin at 30 mg/kg/day, intraperitoneal (IP) seven days before $A\beta_{1-42}$ administration (0.1 μg/μl, 0.5 μl per side, intra-hippocampally (IH)) decreased the amount of mitochondrial ROS formation, mitochondrial lipid peroxidation, and increased glutathione (GSH) level (Yousefsani et al. 2021). Similarly, a14 days treatment with crocin at 10, 20 and 40 m/kg, i.p. meaningfully diminished the malondialdehyde (MDA) level, and at 10 and 20 m/kg, i.p. increased the GSH level in malathion (100 mg/kg)-induced AD in the rat (Mohammadzadeh et al. 2020). Furthermore, a noticeable anti-oxidant activity of crocin was seen in studies by Naghizadeh et al. They detected that intracerebroventricular (ICV) streptozotocin (STZ) on days 1 and 3 (3 mg/kg bilaterally) elevated the MDA concentration and reduced total thiol content and GPx activity, whereas crocin for 21 days (100 mg/kg, p.o.) reversed these effects in rat AD model (Naghizadeh et al. 2013; Naghizadeh et al. 2014). The effects of crocin on tau phosphorylation induced by oral administration of acrolein (3 mg/kg/day) in the cerebral cortex of rats for two weeks were evaluated. The results have shown that crocin significantly inhibited the changes caused by acrolein injection, including a decrease in the level of MDA (at 25 and 50 mg/kg), Aβ (at 25 mg/kg) and phospho-tau (at 25 mg/kg) (Rashedinia et al. 2015). In a recently published paper, it was demonstrated that pretreatment with crocin for 4 weeks at 10, 20, and 30 mg/kg represented therapeutic effects in unpredictable chronic mild stress (UCMS) in rats. Based on the results, crocin reduced oxidative stress induced by UCMS in the AD model via decreasing MDA and nitrite levels and increasing total thiol content, SOD, and catalase (CAT) activity (Abbaszade-Cheragheali et al. 2023). Similarly, crocin (100 mg/kg; i.p.) for 12 days significantly diminished lipid peroxidation in the hippocampus in lipopolysaccharide (LPS)-induced AD in rats in a recent study by Dastan et al. (Dastan et al. 2024).

Excessive ROS generation through oxidation reactions can cause damage to

DNA, RNA, lipid, and protein, which leads to neurodegenerative diseases such as AD and PD and eventually cell death (Lu et al. 2021). However, a broad range of non-enzymatic and enzymatic avoidance mechanisms such as GSH, SOD, GPx, and CAT can control the biological effects of ROS. Also, lipid peroxidation is known as a common outcome of cell death, which can induce DNA and tissue damage (İnal et al. 2001). One of the principal end-products of this process is MDA formed from oxidative stress on biomembranes or lipoproteins (Abuja and Albertini 2001). Overall, crocin could possess effective antioxidant activities and can be used as an antioxidant agent against oxidative stress via decreasing ROS, MDA and acetylcholinesterase levels, and increasing the levels of SOD, GPx, GSH, choline acetyltransferase, and acetylcholine. It seems that in almost studies crocin exert its antioxidant effects by diminishing ROS and MDA levels and increasing the levels of SOD, and GSH.

Anti-inflammatory properties of crocin in AD

Anti-inflammatory effects of crocin in AD were examined in four studies. Mohammadzadeh et al. investigated the effects of crocin for 14 days (10, 20 and 40 m/kg, i.p.) on rats exposed to malathion (100 mg/kg) as an AD model. They found that crocin at 10 m/kg significantly diminished TNF-α and IL-6 levels compared to the malathion group (Mohammadzadeh et al. 2020). Similarly, it was also revealed that IP crocin (30 mg/kg) treatment for 12 days protected against inflammatory effects of $A\beta_{1-42}$ via decreasing the expression of TNF-α and IL-1β mRNA levels in the hippocampus of rats (Hadipour et al. 2021a). In addition, a recent study on UCMS rats as an AD model demonstrated that crocin (10, 20, and 30 mg/kg/day) can reduce TNF-α and IL-10 levels (Rashedinia et al. 2015). Also based on the research results of Sadoughi crocin (25 and 50 mg/kg) administration significantly decreased the TNF-α, IL-1β, and IL-6 in the hippocampus of rats exposed to 8 mg/kg trimethyltin chloride (TMT) as an AD model (Sadoughi 2019). In a recent study Su et al. have suggested that crocin (40 mg/kg; i.p.; for 14 days) revealed anti-inflammatory effects through the significant downregulation of IL-1β, IL-6, and TNF-α mRNA expressions using qRT-PCR in Aβ25-35- induced AD in mice (Su et al. 2024). In addition, in a recent study, Dastan et al. reported that crocin 100 mg/kg (i.p.; 12 days) attenuated the mRNA expression of TNF-α in LPS-induced AD in rats (Dastan et al. 2024).

As mentioned before, interleukin (IL-1β, IL-6) and TNF-α are the main proinflammatory cytokines that can affect the secretion of other inflammatory mediators. These substances can exacerbate the inflammatory process, which is associated with neurodegenerative diseases including AD and PD (Wang et al. 2020). To sum up, crocin exhibits anti-inflammation activity via inhibiting the inflammatory mediators and cytokines such as TNF-α and IL-6, IL-10, and IL-1β levels. It seems that TNF-α is a principal inflammatory marker in almost all reviewed studies.

Cytoprotective properties of crocin in AD

The cytoprotective effects of crocin in AD were evaluated in 10 studies. An investigation was conducted to evaluate the effects of crocin on cell viability, neuronal arborization and cell death in a rat model of AD using a Cresyl violet, Golgi-Cox and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, respectively. The crocin was administered at 30 mg/kg in 12 days. They revealed that crocin significantly augmented the number of live cells and reduced the apoptotic cell number in the CA3 and dentate gyrus (DG) regions of the hippocampus in AD induced by $A\beta_{1-42}$ injection. It also

ameliorated the spine, axonal, and dendrites arborization in the CA3 region and frontal cortex of the rat model of AD (Hadipour et al. 2021b). Similarly, the cytoprotective effect of crocin (30 mg/kg; 12 days) was also observed by diminishing the number of TUNEL-positive cells in the CA1 region and reducing c-Fos in the rat hippocampus as an *in vivo* Aβ model of the AD (Hadipour et al. 2018). In another experiment, crocin (40 mg/kg/day; for 14 days) caused a decrease in apoptotic cell number in prefrontal cortical neurons and hippocampal CA1 region detected by TUNEL staining in $A\beta_{25–35}$-induced AD in rats. Also, the b-cell lymphoma 2 (Bcl-2) expression level significantly increased, whereas the Bcl-2-associated X protein (Bax), Caspase 3, C/EBP homologous protein (CHOP), and glucose-regulated protein 78 (GRP78) expression levels significantly reduced. Moreover, resveratrol exhibited similar anti-apoptotic properties and significantly reversed the expression of these proteins in AD rats and diminished the apoptotic cells (Lin et al. 2019). Another evaluation in 2015 showed that the protective features caused by crocin (150, 300 and 600 nmol) appeared during the down-regulation of the Bax/Bcl-2 ratio and cleaved caspase-3 level in a rat model of AD (Aβ (50 ng/side)) (Asadi et al. 2015). More in-depth studies showed the beneficial effects of crocin in AD. Based on the results, crocin (30 mg/kg/day; i.p. seven days before $A\beta_{1-42}$ administration) ameliorated $A\beta_{1-42}$-induced mitochondrial membrane potential (MMP) decrease, cytochrome c expulsion from mitochondria following mitochondrial swelling as a result of the mitochondrial permeability transition (MPT) pores disturbance caused by $A\beta_{1-42}$, and mitochondrial outer membrane damages induced by $A\beta_{1-42}$ (Yousefsani et al. 2021). In another study, Mohammadzadeh et al. reported that malathion (100 mg/kg) provoked apoptosis by the activation of caspase-3, 8 and 9 cleavages, up-regulation of Bax/Bcl2 ratio as the main apoptotic characteristics in a rat model of AD. They found that crocin i.p. administration (10 mg/kg) significantly reduced the Bax/Bcl2 ratio and cleaved caspase-3, 8 and 9 protein levels, also decreasing TAU mRNA expression (Mohammadzadeh et al. 2020). Also, crocin showed cytoprotective effects via down-regulating p-Akt (protein kinase B) (Akt pathway) (at 12.5-50 mg/kg), and p-extracellular signal-regulated kinase1/2 (ERK1/2) (at 12.5 and 25 mg/kg), and p-stress-activated protein kinase/ c-Jun N-terminal kinase (SAPK/JNK) (at 25 mg/kg) mitogen-activated protein kinase (MAPKs) signaling pathways (Rashedinia et al. 2015). In a recent study, Abbaszade-Cheragheali et al. showed that crocin (10, 20, and 30 mg/kg/day) could decrease Aβ levels in the hippocampus of rats which led to improved memory impairment induced by UCMS (Abbaszade-Cheragheali et al. 2023). In agreement with this finding, crocin (30 mg/kg; i.p, from P14 to P27) was also reported reducing the production of β-amyloid (βA_{42} and βA_{40}), a fragment of amyloid precursor protein (APP) in neonate rat brain on the gestational hypoxia (under 7% O2 + 93% N2 condition for 3 hr; on the 20th day of pregnancy) through decreasing hypoxia-inducible factor-1 (HIF-1) α and β-secretase (BACE1) mRNA level. Based on the results, overexpression of HIF-1α and enhanced BACE1 activity induced by hypoxia can increase the production of β-A (Ghotbeddin et al. 2021). A similar observation was recorded regarding a reduction of β-amyloid production following the down-regulation of HIF-1α and BACE1 mRNA levels in the brain of offspring rats after crocin treatment (Truski et al. 2020). Based on a study conducted with a TMT-induced AD model in rats, administration of crocin (25 and 50 mg/kg) increased Bcl-2, brain-derived neurotrophic factor (BDNF), and neuronal density of CA1, CA2, and CA3 regions and diminished Bax, caspase-9, phosphor tau protein (Pt), and $A\beta_{40}$ levels in the

hippocampus (Sadoughi 2019). Recently, Moghadasi and colleagues documented that crocin supplementation (25 mg/kg; i.p.) and endurance training significantly augmented nerve growth factor (NGF), BDNF, and tyrosine kinase B (TrkB) gene expression and diminished tau gene expression, as well as crocin-endurance training combination presented the most profound these effects (Moghadasi et al. 2024). Additionally, in a recent study, Dastan et al. revealed that 100 mg/kg crocin (i.p.) for 12 days decreased NF-κB and caspase 3 mRNA expression in LPS-induced AD in rats (Dastan et al. 2024).

One of the most important apoptosis pathways is the intrinsic or mitochondrial pathway that gives rise to the release of cytochrome-c from the mitochondria to the cytosol and leads to augmenting the death signal. Release of cytochrome-c following a proapoptotic protein activation subsequently triggers the caspase cascade such as caspase-3, and 9 and leads to cell death (Ghobrial et al. 2005). Evidence shows crocin has cytoprotective properties to inhibit apoptosis via decreasing cytochrome c expulsion from mitochondria and mitochondrial outer membrane damages, Bax/Bcl2 ratio, cleaved protein level, and down-regulating p-Akt, p-ERK1/2, and p-SAPK/JNK levels. It seems that the Bax/Bcl2 ratio and caspase-3 are the principal apoptosis markers in almost all reviewed studies. In addition, it appears that the PI3K/Akt/mTOR signaling pathway as a critical cell signaling involved in cell growth, and metabolisms, is very impressive in exploring the neuroprotective properties of crocin.

There was no clinical study of crocin effects in AD patients. However, a comprehensive study reported that saffron and its main constituent crocin augmented learning-memory, sleep quality (saffron (0.6 mg/day)), and decreased neuronal cell death, depression and sleep disorders (saffron (100 mg–1 g/day)) through anti-oxidant and anti-inflammation activities. It is worthy to mention that sleep disturbance may increase inflammatory processes, which leads to dementia and Alzheimer's disease (Kuchta et al. 2022).

In vivo neuroprotective mechanisms of crocin in PD

There is a noticeable body of evidence that concerns the neuroprotective effects of crocin in different *in vivo* experimental models and we summarized the studies which include rat/mice models of 6-hydroxydopamine (6-OHDA)-induced-Parkinson's disease. Based on the results of these *in vivo* studies, crocin exhibited neuroprotective effects in PD via anti-oxidant, anti-inflammatory and cytoprotective properties. Overall crocin exhibited significant effects in preventing molecular signaling evoked by neural insults and exerting neuroprotection. The molecular mechanisms are discussed in the following part (Table 3 & Figure 2).

Anti-oxidant properties of crocin in PD

Anti-oxidant action of crocin in PD was evaluated in eight studies. Rats treated with crocin (10, 20, and 40 mg/kg) for 28 days were protected against malathion (100 mg/kg; i.p.)-induced Parkinson-like behaviours in aspects of oxidative stress via decreasing MDA and increasing GSH levels. Interestingly, they demonstrated that crocin or levodopa (10 mg/kg/day, i.p.) showed similar efficacy in the striatum in malathion (100 mg/kg/day, i.p.)-induced PD model (Mohammadzadeh et al. 2018). In another PD model study, crocin at 60 mg/kg for six weeks ameliorated 6-OHDA (16 μg in 0.2% ascorbate-saline)-induced nitrosative damage and significantly lessened the nitrite levels in the striatum in hemiparkinsonian Rats (Hosseini et al. 2016). Several studies have shown that reactive nitrogen species (RNS), such as peroxynitrite and nitric oxide (NO) play a crucial role in dopaminergic neuron toxicity in PD. The particular role of 6-OHDA in the induction of inducible nitric

oxide synthase (iNOS) expression and nitrite levels that led to dopaminergic cell toxicity has been confirmed in a variety of studies (Di Matteo et al. 2009; Singh et al. 2005; Singh et al. 2010). Similarly, crocin for six weeks at 30 and 60 mg/kg decreased thiobarbituric acid reactive substances (TBARS) and nitrite levels in the hippocampus in 6-OHDA (16 μg in 0.2% ascorbate-saline)-induced PD in rats (Rajaei et al. 2016). Shahidani and colleagues showed that crocin (100 mg/kg, for six weeks) exerts its effects by lessening the lipid peroxidation levels in the hippocampus in 6-OHDA (16 μg)-induced PD in rats (Shahidani et al. 2019). It has been reported that crocin treatment noticeably mitigated ROT-induced toxicity in rat models of PD. Crocin for 4 weeks at doses of 20 and 40 mg/Kg/day revealed a reduction in MDA, serum 8-hydroxydeoxyguanosine (8-OHdG), and nitrite/nitrate levels and also increased GSH and dopamine levels in ROT-induced PD in the rat. Similarly, in this study, levodopa displayed these antioxidant effects (Abeer and Ahmed 2019). In a study, Salama and colleagues reported that 30 mg/kg/day, i.p. for 30 days of crocin significantly increased tyrosine hydroxylase (TH) level in rat exposure to ROT (1.5 mg/kg/day, i.p.) as a PD model (Salama et al. 2020). A similar study indicated that crocin (30 mg/kg for 15 days) could exert beneficial effects by increasing the number of TH-positive neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (30 mg/kg for 5 days)-induced PD in mice (Haeri et al. 2019). Promotion in the expression of TH in the medial prefrontal cortex (mPFC) and ventral tegmental area (VTA) of MPTP (30 mg/kg)-induced mouse model of PD after administration of crocin for 7 days (after MPTP administration) was also evident (Tang et al. 2020). Crocin has potent antioxidant activities and seems it have the potential to combat oxidative stress via reducing MDA, GSH, nitrite lipid peroxidation, 8-OHdG levels, and increasing the number of TH-positive neurons.

Anti-inflammatory properties of crocin in PD

The anti-inflammatory activities of crocin in PD were tested in three studies. Mohammadzadeh and her colleagues have shown that 10 mg/kg of crocin diminished TNF-α and IL-6 levels in the striatum in malathion (100 mg/kg/day, i.p.)-induced Parkinson-like injury in rats (Mohammadzadeh et al. 2018). Also, a significant decrease in TNF-α levels in the striatum in hemiparkinsonian rats induced by 6-OHDA (16 μg) has been reported following administration of 100 mg/kg crocin, for six weeks (Shahidani et al. 2019). Similarly, it was also shown that crocin (20 and 40 mg/Kg/day for 4 weeks) treatment significantly decreased TNF-α levels in ROT-induced PD in rats (Abeer and Ahmed 2019). Similarly, in a recent study, Alizadehmoghaddam et al. documented that 15 and 30 mg/kg crocin intraperitoneally for 21 days reduced the number of caspase-1 and IL-1β positive cells, IL-1β, IL-18, NLRP1, and AIM2 genes expression also increased TH expression in LPS-induced PD in rat (Alizadehmoghaddam et al. 2024).

Cytoprotective properties of crocin in PD

Numerous research projects have established that crocin exerts protective effects in PD via cytoprotective properties. Based on the results, the cytoprotective effects of crocin in PD were assessed in four studies. The protective effects of crocin (30 mg/kg/day; i.p.) on a rat model of PD after exposure to ROT (1.5 mg/kg/day, i.p.) for 30 days. Based on the results i.p. administration of crocin considerably stimulated phosphoinositide 3-kinases (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway and increased miRNA-7 and miRNA-221 expression level as activators of Akt/mTOR. It also weakened caspase-9,

forkhead box transcription factor of the O class (FoxO3a), glycogen synthase kinase-3β (GSK-3β), and α-synuclein level and increased dopamine level (Salama et al. 2020). Similarly, Haeri et al. found that crocin (30 mg/kg for 15 days) significantly increases the number of dark neurons and decreases cell death in the substantia nigra in MPTP (30 mg/kg for 5 days)-induced PD in mice (Haeri et al. 2019). Mohammadzadeh and colleagues revealed that administration of crocin (10 mg/kg/day, i.p.) for 28 days shows its effects via lessening Bax/Bcl2 ratio, caspases-3, 9 protein levels, and mRNA level of α-synuclein in striatum tissue of rat exposed to malathion (100 mg/kg/day, i.p.) as a PD model. Interestingly they found that co-administration of crocin or levodopa (10 mg/kg/day, i.p.) suppressed the neurotoxic effects of malathion on striatal tissue via anti-apoptotic activities (Mohammadzadeh et al. 2022). The observations of another study showed that crocin for 7 days could significantly diminish the symptoms related to Parkinson's disease depression (PDD) in the VTA of the MPTP (30 mg/kg/day)-treated mice through improving the mTOR signaling pathway (Tang et al. 2020). Overall, crocin displays cytoprotective effects through enhancing PI3K/Akt/mTOR signaling pathway activity and decreasing GSK-3β, FoxO3a, and caspase-9 levels Bax/Bcl2 ratio.

Interestingly in some clinical studies crocin showed neuroprotective effects in PD patients. In a study, Soleymani et al. explored the crocin effects on movement disorders and neuronal oxidative DNA damage in PD patients. They found that crocin (30 mg twice daily; before and after an 8-week intervention) improved daily life activities and alleviated movement disorders in patients with idiopathic PD (Soleymani et al. 2024).

In this systematic review, we have reviewed 28 high-quality animal studies on the neuroprotective effects of crocin against AD and PD. Overall, crocin is revealed to possess beneficial therapeutic properties and is a potential agent for the treatment/slowing the process of AD and PD. According to the systematic evaluation of efficacy and pharmacologic mechanisms, the administration of crocin enhances cell viability as well as anti-oxidant activities via a decrease in the biomarkers of oxidative stress, an increase in the anti-oxidant enzyme levels and mitochondrial enzyme function. Based on the results of research studies, reducing ROS, MDA, GSH, 8-OHdG, acetylcholinesterase, and nitrite lipid peroxidation levels, and increasing the number of TH-positive neurons, SOD, GPx, GSH, choline acetyltransferase, and acetylcholine levels indicate the anti-oxidant effects of crocin. Also, crocin can reduce inflammatory responses with various mechanisms including a decrease in TNF-α and IL-6, IL-10, and IL-1β levels. In almost studies TNF-α as a principal inflammatory marker were reported. Crocin can augment the cytoprotective cellular defenses by affecting the main apoptotic mediators and main apoptotic signaling pathways. The proposed important mechanism for the cytoprotective effects of crocin is reduction of GSK-3β, FoxO3a, cleaved caspase-3, 8 and 9 levels, Bax/Bcl2 ratio, cytochrome c expulsion from mitochondria and mitochondrial outer membrane damages, as well as down-regulation p-Akt, p-ERK1/2, and p-SAPK/JNK levels, and augmentation PI3K/Akt/mTOR signaling pathway activity. In almost all studies Bax/Bcl2 ratio and caspase-3 as principal apoptosis markers were described. Since the PI3K/Akt/mTOR signaling pathway plays a critical role in regulating cell growth, cell survival, and cell proliferation, it seems that exploring this pathway in the neuroprotective properties of crocin is very effective and important. It has been reported that the potent effective dose of crocin in *in vivo* studies was 10-100 mg/kg, i.p. (average of 30-50 mg/kg, i.p.) and 150-600 nmol.

Although the anti-oxidant, anti-inflammatory and cytoprotective effects of crocin in most studies follow their similar mechanisms as discussed above, it is suggested that a low dose of crocin 30-50 mg/kg displayed the best neuroprotective effects however, there was also proposed that a high dose of crocin up to 100 mg/kg does not impact on preventing AD and PD process. It has been indicated that the justifiable route of administration of crocin in *in vivo* studies was i.p. because it is considered as a potent route for pharmacological studies to assess the properties of target interactions. Considering the confident results from animal studies and the relatively low toxicity of crocin, we need more clinical trials in human subjects to evaluate and ensure the efficacy of crocin in reducing neurological damage in patients suffering from AD and PD. However, based on the mentioned studies, some limitations have been observed in this way. First of all, the use of different mentioned doses of crocin was not examined based on concentration. Also, the technique for administration of crocin varied among the studies from intragastric to intraperitoneal and intracerebroventricular injections. Two notable limitations were that different substances or neurotoxins with various induction duration were utilized to create an AD and PD model in animals. In addition, the study's scope is limited by the time, language and database restrictions because of the numerous studies on the neuroprotective effects of crocin all over the world especially in Iran. Considering that the number of reports regarding the neuroprotective effects of crocin is increasing, this systematic review has investigated high-quality animal studies on the anti-AD and anti-PD effects of crocin. Also, most of the studies were evaluated as low risk for sequence generation, baseline characteristics, allocation concealment, random housing, incomplete outcome data, selective outcome reporting, and other sources of bias. However, most studies are unclear for blinding in bias, random outcome assessment and blinding in detection bias. The chance of bias could impact on inference of the results and limit the study's scope. Based on the results, crocin shows several biological functions such as antioxidant, anti-inflammatory and cytoprotective and displays potential therapeutic effects in *in vivo* models of AD and PD. While the effects of crocin *in vivo* models of AD and PD are clear, the exact mechanisms are not fully clarified. There are incomplete molecular mechanisms and information on the underlying biological activities of crocin. To overcome this obstacle, the following suggestion is ultimately recommended; more research is needed to focus on investigating new probable molecular mechanisms to provide more insightful information for future clinical trials for slowing the process or treating AD and PD.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

Author contributions

Zahra Tayarani-Najaran: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing - Review & Editing. Elham Hadipour: Writing - Review & Editing. Shirin Ramazani and Leyli Taghizadeh Methodology, Validation, Investigation, Formal analysis, Writing the original draft. Elham Ramazani: Conceptualization, Methodology, Validation, Investigation, Formal analysis, Funding acquisition, Project administration, Writing - Review & Editing. All authors reviewed, considered, and approved the manuscript.

Abbreviations

Aβ, Amyloid beta; AChEIs, Acetylcholinesterase inhibitors; AD, Alzheimer's disease; Akt, Protein kinase B; APP, Amyloid precursor protein; BACE1, β-secretase; Bax, Bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; BDNF, Brain-derived neurotrophic factor; CAT, Catalase; CHOP, C/EBP homologous protein; CPC, Centrifugal partition chromatography; DBS, Deep brain stimulation; DG, Dentate gyrus; ERK1/2, Extracellular signal-regulated kinase 1/2; FoxO3a, Forkhead box transcription factor of the O class; GPx, Glutathione peroxidase; GRP78, Glucose regulated protein 78; GSH, Glutathione; GSK-3β, Glycogen synthase kinase-3β; HIF-1, Hypoxia-inducible factor-1; HPLC, High-performance liquid chromatography; HPTLC, High performance thin layer chromatographic; ICV, Intracerebroventricular; IH, Intra-hippocampally; IL, interleukin; iNOS, Inducible nitric oxide synthase; IP, Intraperitoneal; ITM, Islamic Traditional Medicine; JNK, c-Jun N-terminal kinase; LPS, Lipopolysaccharide; MAPKs, Mitogen-activated protein kinase; MDA, Malondialdehyde; MISPE, Molecular imprinting solid phase extraction; MMP, Mitochondrial membrane potential; mPFC, Medial prefrontal cortex; MPLC, Medium pressure liquid chromatography; MPT, Mitochondrial permeability transition; MPTP, 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mTOR, Mammalian target of rapamycin; NACE, Non-aqueous capillary electrophoresis; NFTs, Neurofibrillary tangles; NGF, Nerve growth factor; NMDAR, N-methyl-D-aspartate receptor; NO, Nitric oxide; 6-OHDA, 6-Hydroxydopamine; 8-OHdG, 8-Hydroxydeoxyguanosine; PD, Parkinson's disease; PI3K Phosphoinositide 3-kinases; PrepTLC, Preparative thin layer chromatography; Pt, Phosphor tau protein; RNS, Reactive nitrogen species; ROS, Reactive oxygen species; ROT, Rotenone; SAPK, Stress-activated protein kinase; SN, Substantia nigra; SOD, Superoxide dismutase; STZ, Streptozotocin; TBARS, Thiobarbituric acid reactive substances; TH, Tyrosine hydroxylase; TrkB, Tyrosine kinase B; TMT, Trimethyltin chloride; TNF-α, Tumour necrosis factor alpha; TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling; UCMS, Unpredictable chronic mild stress; VTA, Ventral tegmental area.

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