Active constituents of saffron (*Crocus sativus* L.) and their prospects in treating neurodegenerative diseases (Review)

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Abstract. *Crocus sativus* L. (saffron) is widely used as a traditional spice for flavoring, coloring and medicinal purposes. As a traditional Chinese herb, saffron promotes blood circulation, removes blood stasis, cools and detoxifies the blood, relieves depression and calms the mind. According to modern pharmacological studies, the active constituents of saffron, including crocetin, safranal and crocus aldehyde, exhibit antioxidant, anti-inflammatory, mitochondrial function-improving and antidepressant effects. Thus, saffron has the potential to treat neurodegenerative diseases (NDs) associated with oxidative stress, inflammation and impaired mitochondrial function, such as Alzheimer's disease, Parkinson's disease, multiple sclerosis and cerebral ischemia. The present article provides a review of the pharmacological effects of saffron and its constituents in terms of neuroprotective effects, including antioxidant and anti-inflammatory effects and the improvement of mitochondrial dysfunction, as well as their clinical application in treating NDs.

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Abbreviations: AChE, acetylcholinesterase; AD, Alzheimer's disease; Aβ, amyloid β; BDNF, brain-derived neurotrophic factor; BI, brain injury; CHOP, C/EBP homologous protein; CI, cerebral ischemia; CR, crocin; ERK, extracellular regulated protein kinase; GSH-Px, glutathione peroxidase; IL, interleukin; MAPK, mitogen-activated protein kinase; MDA, malondialdehyde; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; mPTP, mitochondrial permeability transition pore; MS, multiple sclerosis; ND, neurodegenerative disease; OS, oxidative stress; PD, Parkinson's disease; PSD, post-stroke depression; ROS, reactive oxygen species; SOD, superoxide dismutase; STZ, streptozotocin

Key words: neurodegenerative disease, *Crocus sativus* L., crocin, safranal, Alzheimer's disease, Parkinson's disease

Contents

1. Introduction

Saffron, the dried stigmas of the flowers of *Crocus sativus* L., is able to promote blood circulation and prevent blood stasis, cool and detoxify the blood, relieve depression and calm the mind (1). According to textual research, saffron was introduced to China during the Ming Dynasty (AC1368-1644) and had a long history of medicinal use (2). According to the Compendium of Materia Medica, saffron has been mainly used to treat palpitations, promote blood circulation and relieve depression or restlessness (3). In recent years, the constituents of saffron and pharmacological activities have been extensively studied in China and abroad. A total of >150 compounds have been identified in saffron (4), including terpenoids, anthraquinones, amino acids and alkaloids. Terpenoids, the most abundant compounds in saffron, include crocin (CR), safranal, picrocrocin and crocetin (structural formulas are provided in Fig. 1), of which the first three are responsible for the color, aroma and bitterness of saffron. Their contents

are crucial indexes to measure the quality of saffron in various national quality standards, and the pharmacopoeias of numerous countries have clear regulations on the content of crocin and bitter picrocrocin (5). Saffron may be used to prevent and treat neurodegenerative diseases (NDs) due to its pharmacological effects, including the inhibition of atherosclerosis (6) and platelet aggregation (7), blood lipid reduction (8), antioxidant function (9), improvement of myocardial ischemia and hypoxia (10), anti-inflammatory (11,12), anticancer (13) and antidepressant functions (14). In addition to being used as a medicine, saffron is also sold as a food product. The application of saffron as a spice in the food industry is one of its numerous uses, as its unique fragrance stimulates the taste buds and increases appetite (15). Furthermore, numerous researchers have purified its active constituents and incorporated them into nutraceutical additives to prevent chronic diseases such as cancer and cardiovascular and neurodegenerative diseases (16,17). However, its high price has prevented its widespread use.

ND is a type of disease in which cells and neurons of the brain and spinal cord are lost, which is caused by the loss of neurons or their myelin sheaths (18), eventually leading to dysfunction. NDs include chronic diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and multiple sclerosis (MS), as well as acute diseases, such as cerebral ischemia (CI) and brain injury (BI). NDs may be related to oxidative stress (OS), protein aggregate deposition, neuroinflammation, impaired mitochondrial function, apoptosis induction and autophagy changes (19,20). Extensive research on the mechanism and treatment of NDs has been conducted; however, the pathogenesis of these diseases remains to be fully elucidated. As there is currently no definite cure for NDs, most cases require conservative treatment, but conservative treatment with medications may be associated with gastrointestinal responses (such as nausea and vomiting), cardiovascular reactions (including tachycardia and arrhythmia) and mental disorders (such as anxiety and depression) (21,22).

Saffron and its active constituents (mainly CR, safranal and crocetin) have potent antioxidant and anti-inflammatory effects on brain cells, prevent amyloid β (Aβ) aggregation and regulate the steady-state concentration of metal ions in the brain (23,24). Thus, saffron has therapeutic potential for AD (25,26), PD (27) MS (28) and CI (29), and may reverse neurotoxicity caused by toxic substances, thereby protecting neurons (30,31). Furthermore, ND is usually accompanied by depressive symptoms. A previous study indicated that 36.23% of patients with PD suffer from depression, whereas 68.42% suffer from anxiety (32). Thus, due to its anti-depressant and anxiolytic properties, saffron may effectively treat depressive symptoms. Based on these pharmacological effects, saffron has a reasonable prospect as an auxiliary drug for ND treatment.

In the last decade, the pharmacological action of saffron for ND treatment has been extensively studied in animal models. However, the available reviews do not provide comprehensive summaries on the use and mechanism of action of saffron for the treatment of ND. The pathogenesis of ND is relatively complex (33,34). To achieve a better therapeutic effect, the pathway of saffron action must be further clarified. The present article reviewed the research progress on the use of saffron extract and its active constituents for ND treatment, particularly the pharmacological experiments performed in animal models of AD, PD, MS and CI, to comprehensively summarize the research results and administration methods of saffron in this field, focusing on its pharmacological effects during treatment. Up-to-date information on the potential mechanism by which saffron exerts neuroprotection through pharmacological activities and its therapeutic prospects in NDs is presented.

2. Methods

The literature was screened by WY and XQ. The Chinese National Knowledge Infrastructure, PubMed, ScienceDirect, ACS publication, Scopus and Medline databases, as well as Wiley Online Library, were searched for articles published in the Chinese and English languages, mainly referring to the literature from 1987 to 2022. The search terms mainly included crocin, safranal and other active constituents of saffron, as well as neurodegenerative disease,s such as AD and PD, which are terms that are commonly used in the pharmaceutical industry. The pharmacological research related to saffron was primarily distributed over the last decade. The final list of included studies was approved by MZ and JP.

3. Antioxidant effect of saffron and its active constituents

Aβ and Tau are associated with OS in AD. OS is caused by free radicals, atoms or groups with unpaired electrons, such as hydroxyl, superoxide and nitric monoxide (35). OS may cause damage to the cells and tissues of the body, such as the DNA, RNA, protein and lipid bilayer of nerve cells (36-38). Oxidative damage to nerve tissue has been found in NDs, such as AD, PD and amyotrophic lateral sclerosis. Studies have indicated a close association between Aβ, Tau protein and OS in neurons. For instance, Aβ aggregation may damage the mitochondria, leading to mitochondrial dysfunction and the release of numerous reactive oxygen species (ROS) and OS; the generation of ROS may also increase the production of Aβ (39,40). Free radicals, such as oxidized Fe^{3+}, may promote the phosphorylation and aggregation of Tau (41). Furthermore, hyperphosphorylation and accumulation of Tau may also damage mitochondrial function, thereby generating a substantial amount of ROS. Chronic OS and the resultant peroxides, such as 4-hydroxynonenal, may also provoke Tau hyperphosphorylation, causing conformational changes and Tau aggregation (42). Aβ aggregation and Tau hyperphosphorylation have crucial roles in the pathogenesis of AD (43-46). In addition, OS is also associated with the free iron content of cells and elevated levels of iron ions may be found in the brains of patients with PD or AD (47). Therefore, it may be concluded that OS is intertwined with ND.

Inhibitory effects of saffron on Aβ aggregation and Tau abnormal phosphorylation. One of the main pathological features of AD is the formation of senile plaques by the accumulating Aβ outside the brain nerve cells. CR, the major active component of saffron, has been indicated to increase the tightness of a cell-based blood-brain barrier model, increase recombinant low-density lipoprotein receptor-related protein 1

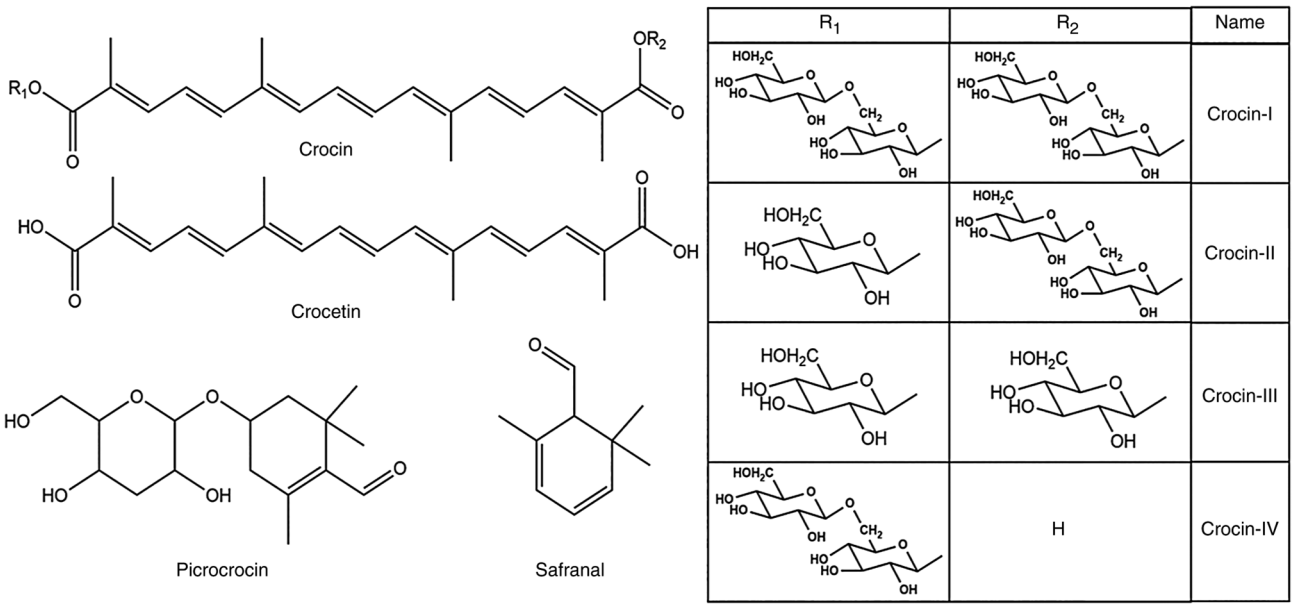

Figure 1. Structural formula of main active components of saffron. Crocin-I: R1=R2=gentobiosyl-; crocin-II: R1=β-D-glucosyl-, R2=D-gentobiosyl-; crocin-III: R1=R2=β-D-glucosyl-; crocin-IV: R1=β-D-gentobiosyl-, R2=H.

and P-glycoprotein expression, improve Aβ clearance, reduce Aβ aggregation and inhibit the formation of senile plaques (48). Both CR and the extract of saffron (water/methanol, 50:50 v/v) was reported to inhibit the accumulation of Aβ in the human brain through antioxidant effects (49). Furthermore, saffron extract was able to antagonize aluminum oxide-induced neurotoxicity by elevating the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px) (50). Streptozotocin (STZ) may cause OS by increasing the production of oxygen-free radicals, thereby inducing cognitive impairment (51). In addition, CR may elevate GSH-Px activity and the total thiol content and reduce malondialdehyde (MDA) levels and OS damage, producing an antagonistic effect on STZ-induced cognitive impairment in rats (52-54). CR may also significantly decrease the Bax/Bcl-2 ratio and cleaved caspase-3 levels by reducing ROS production and inhibiting Aβ-induced apoptosis (55,56). Mitogen-activated protein kinases (MAPKs) are serine-threonine kinases that mediate intracellular signaling related to various cellular activities, including cell proliferation, differentiation and transformation (57). When OS is triggered, ROS activates downstream apoptosis pathways through the MAPK pathway, such as NF-κB and p53, triggering cell death (58-60). Safranal may reduce the ROS levels in cells, thereby reducing Aβ-induced apoptosis through the PI3K/AKT and MAPK/extracellular regulated protein kinase (ERK)1/2 pathways (61). Following treatment of AD rats with safranal, the hippocampal levels of MDA, ROS and protein carbonyl were observed to be reduced, while the activity of SOD and myeloperoxidase was increased in the hippocampal tissue (62).

Abnormal Tau phosphorylation is also one of the leading causes of AD. In the brain of patients with AD, abnormally phosphorylated tau protein has been observed, which, unlike normal tau protein, does not bind to microtubule proteins; instead, its presence also prevents the latter from promoting the assembly of tubulin into microtubules, leading to neurofibrillary tangles (63). Therefore, decreasing Tau hyperphosphorylation is an effective way to treat AD. Trans-CR 4 and trans-crocetin were selected to treat two AD neuronal cell culture models and the results demonstrated that these two compounds did not affect the viability of neuron-like cells. Both trans-CR 4 and trans-crocetin exerted a crucial effect to inhibit amyloidogenic pathways and were influential in suppressing the active forms of ERK1/2 kinases and glycogen synthase kinase-3β, as well as markedly reducing Tau phosphorylation (64). Furthermore, CR was able to significantly decrease MDA, Aβ and phosphorylated Tau levels by modulating the MAPK signaling pathway (65). AD is also characterized by abnormal Tau aggregation, whereas CR is able to inhibit Tau aggregation and suppress the formation of Tau protein filaments (66). Fig. 2 illustrates the relationship between saffron components, Aβ aggregation and Tau abnormal phosphorylation in AD.

Antioxidant effects on PD. In addition to AD, saffron also improves PD symptoms through its antioxidant effects. CR has a protective effect in terms of reducing mitochondrial permeability transition pore-induced dopaminergic neuron damage and PD complications, in addition to ameliorating 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD complications and reducing substantia nigra cell death (67). It was reported that the antioxidant capacity of saffron contributes to PD treatment. After validating the neuroprotective efficacy of methanolic saffron extract and its active constituent, CR, in a drosophila model of Parkinson's disease, studies have proposed that saffron may be used as a complementary therapeutic agent for PD-mediated NDs (68,69). While common NDs, such as AD and PD, have been found to cause oxidative damage in neuronal tissues, saffron and its active compounds may reduce oxidative stress by inhibiting Aβ aggregation, tau protein phosphorylation or ROS production, proving that saffron has considerable

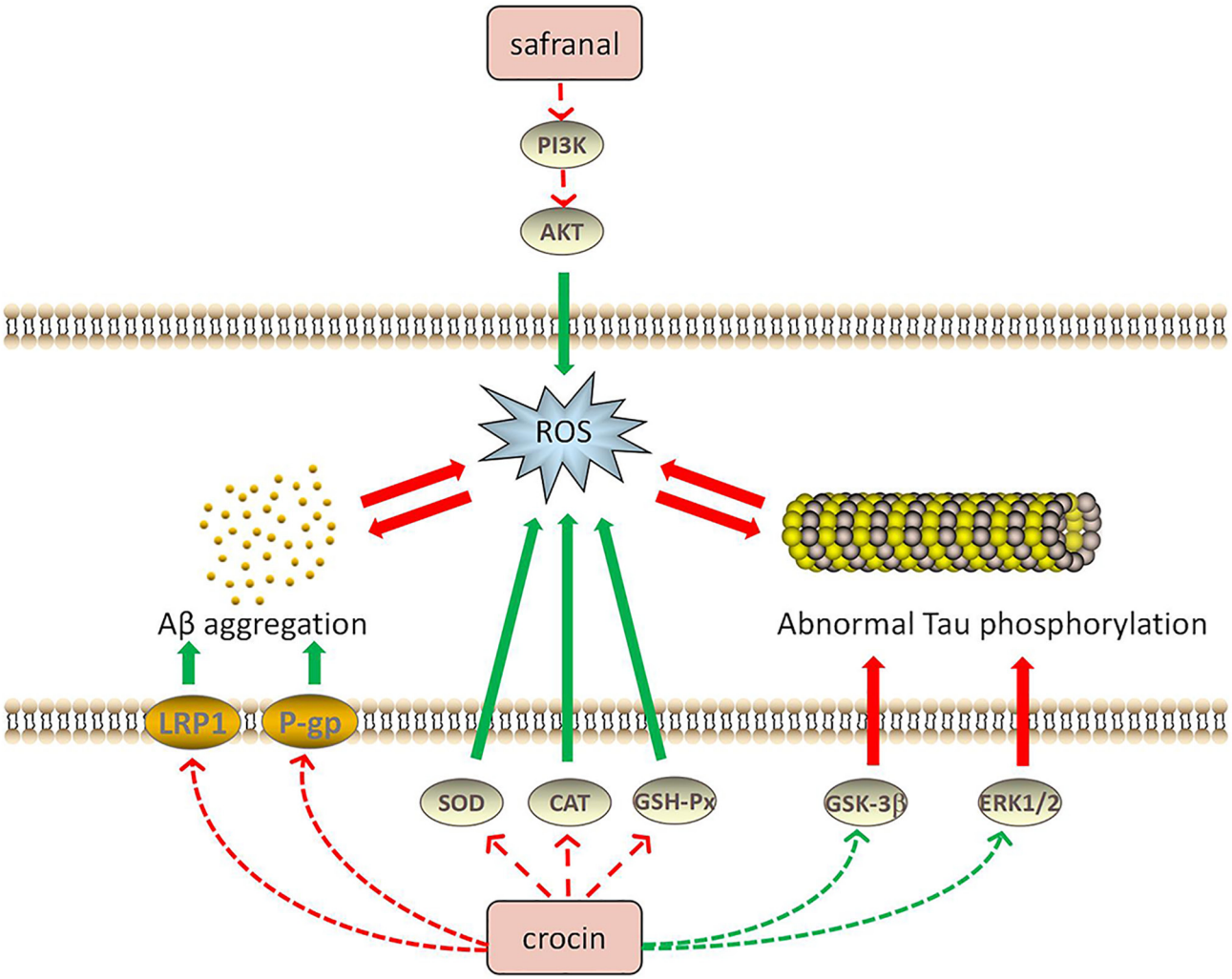

Figure 2. Potential mechanisms of the antioxidant effects of saffron compounds in treating AD. Aβ and Tau proteins are closely associated with oxidative stress. Saffron compounds may reduce oxidative stress by regulating the activity of related proteins to inhibit Aβ aggregation, Tau phosphorylation or ROS production, thereby exerting therapeutic effects on neurodegenerative diseases. Red arrows indicate upregulation and green arrows indicate downregulation of the indicated pathway or factor. PI3K, phosphatidylinositol 3-kinase; AKT, protein kinases B; ROS, reactive oxygen species; AD, Alzheimer's disease; LRP1, recombinant low density lipoprotein receptor-related protein 1; P-gp, P-glycoprotein; SOD, superoxide dismutase; Aβ, amyloid β; CAT, catalase; GSH-Px, glutathione peroxidase; GSK3β, glycogen synthase kinase-3β; ERK, extracellular regulated protein kinase.

therapeutic potential for NDs. Table I presents the relevant research progress regarding the use of saffron to treat NDs through its antioxidant effects.

4. Anti-inflammatory capacity of saffron and its active constituents

Pathological process of neuroinflammation. OS has an essential role in the pathological changes of NDs, whereas neuroinflammation is also crucial for ND pathogenesis (70). Neuroinflammation is a defense mechanism that protects the central nervous system (CNS) from tissue damage or viral attack (71). However, a continuous inflammatory process in the CNS may inflict severe damage to the nervous system and eventually lead to CNS damage (72). Inflammation-derived ND is a specific CNS damage disease. The primary hallmark of inflammation in the brain is the activation of glial cells, particularly microglia, and ROS is involved in microglial activation (73). Microglia cells influence the NF-κB signaling pathway. Tumor necrosis factor-α (TNFα) and interleukin (IL)-1β are common cytokines secreted by activated glial cells (74).

Anti-inflammatory effects of saffron in treating AD and CI. Studies have demonstrated the therapeutic potential of saffron and its active constituents for neuroinflammation-mediated NDs. Saffron extracts were able to upregulate the synaptic proteins in the brains of 5XFAD mice, transgenic mice transfected with five Familial Alzheimer's disease mutations, and reduce Aβ pathology-related neuroinflammation (48). In AD mice, safranal not only reduced the expression of NF-κB and its downstream TNFα, IL-6, apoptosis markers and glial fibrillary acidic protein, but also elevated the mitochondrial membrane potential (Δψm) (62). Safranal exerts anti-inflammatory effects by inhibiting the classic NF-κB inflammatory pathway, thereby improving AD (75). Safranal also reduces the hyperactivity of acetylcholinesterase (AChE) and inhibits cholinesterase overexpression. Furthermore, crocetin may suppress NF-κB activation and P53 expression in the

Table I. Possible mechanisms of action of saffron in the treatment of neurodegenerative diseases through antioxidant effects.

Experimental animal	Model	Administration	Dose	Mechanism of action and target	(Refs.)
Mouse	Transgenic mice transfected with five FAD mutations	Feed with crocin and *Crocus sativus* extract-enriched diet	10 mg/kg crocin; 50 mg/kg crocus sativus extract	Synaptic protein expression ↑, Aβ load ↓, P-gp, LRP1 ↑	(48)
Rat	STZ-induced diabetes	Intraperitoneal injection of saffron extract	200 mg/kg	SOD, CAT, GSH-Px activity↑	(53)
Rat	STZ-induced cognitive impairment	Intracerebroventricular injection of crocin	100 mg/kg	MDA level ↓, GSH-Px activity ↑, TSH content ↑	(54)
Rat	Memory deficit	Peptide injection of crocin	150, 300, 600 nmol/side via IH	Bax/Bcl-2 ratio ↓, cleave Caspase-3 level ↓	(55,56)
Mouse	D-galactose and $AlCl_3$-induced AD	Intraperitoneal injection of crocin	5, 20 mg/kg	Rate of apoptosis ↓, mitochondrial dysfunction ↓, ROS ↓, Ca^{2+} overload ↓, Bax/Bcl-2 ratio, cleaved caspase-3 levels ↓, Aβ 1-42 deposition in the hippocampus ↓, GSH-Px, SOD, ChAT activity ↑	(55,56)
PC12 cell	Aβ and H_2O_2 induced toxicity and oxidative damage	Culture in medium containing saffron	2.5 μM	ROS ↓, apoptosis induced by the PI3K/AKT and MAPK/ERK1/2 pathways ↓	(61)
Rat	$A\beta_{1\text{-}40}$-induced AD	Microinjection of safranal	0.2 ml/kg	MDA, protein base, ROS ↓, SOD, MPO ↑	(62)
SH-SY5Y and PC12 neuronal cells	Overexpressing hyperphosphorylated tau	Exposure to trans-crocin-4 or trans-crocetin concentrations	0.1 μM-1 mM	β secretase (BACE1) ↓, γ secretase (PSEN1/PSEN2 complex) ↓, GSK3 and ERK1/2 kinase activity ↓, sAPPα, sAPPβ ↓	(64)
Rat	Acrolein-induced neurotoxicity	Intraperitoneal injection of crocin	12.5 mg/kg, 25 mg/kg, 50 mg/kg	MAPK signaling pathway, MDA, Aβ, phosphorylated Tau levels ↓	(65)
Drosophila	ROT-induced PD	Maintained on SME or CR-enriched medium	SME: 0.05%, 0.1%; CR: 0, 25 μM	ROS, HP, NO, protein carbonyl ↓, SOD, GST, GSH, TSH ↑, TR, AchE ↓	(68)

↑ indicates elevation or promotion, while ↓ means downregulation or inhibition. AD, Alzheimer's disease; STZ, streptozotocin; Aβ, amyloid β; PD, Parkinson's disease; ROT, rotenone; CR, crocin; P-gp, P-glycoprotein; LRP1, recombinant low density lipoprotein receptor related protein 1; SOD, superoxide dismutase; CAT, catalase; LRP1, recombinant low density lipoprotein receptor related protein 1; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; TSH, thyroid stimulating hormone; Bax/Bcl-2, B-cell lymphoma 2; ROS, reactive oxygen species; ChAT, choline acetyltransferase; PI3K, phosphatidylinositol 3-kinase; AKT, protein kinases B; ERK, extracellular regulated protein kinases; MPO, myeloperoxidase; GST, glutathione S-transferase; GSH, glutathione; sAPP, soluble amyloid precursor protein.

hippocampus, significantly decreasing the pro-inflammatory cytokine secretion and increasing anti-inflammatory cytokine levels in plasma, while inhibiting apoptosis and decreasing Aβ in various brain areas (76).

Acute NDs, such as CI, are also affected by neuroinflammation, a crucial pathological process in the later stage of CI (77). It has been indicated that inflammation mediates CI-reperfusion injury. Ischemic stroke may cause depression, which is a severe disease inflicted by CI, and post-stroke depression (PSD) is a severe complication of stroke (78). It has been demonstrated that persistent CI leads to PSD (79). Thus, PSD is closely associated with inflammation. CR was able to inhibit the inflammatory response by inhibiting the activation of the Toll-like receptor 4/myeloid differentiation

Table II. Possible mechanisms of action of saffron in the treatment of neurodegenerative diseases through anti-inflammatory effects.

Experimental animal	Model	Administration	Dose	Mechanism of action and target	(Refs.)
Mouse	Transgenic mice transfected with five FAD mutations	Feed with *Crocus sativus* extract-enriched diet	50 mg/kg	Synaptic protein expression ↑, Aβ load ↓, P-gp, LRP1 ↑	(48)
Rat	$A\beta_{1-40}$-induced AD	Microinjection of safranal	0.2 ml/kg	IL-1β, 1L-6, TNFα, GFAP, MPO ↓, NF-κB ↓	(62)
Mouse	APPsw transgenesis induced AD	Oral crocetin	0, 10 and 30 mg/kg	Insoluble Aβs secretion ↓, TNFα, IL-1β, IL-8 and IL-6 ↓, IL-10 ↑, NF-κB activation and p53 expression ↓	(76)
Rat	Cerebral stroke	Intraperitoneal injection of crocin	50 mg/kg	Inhibition of the TLR4/MyD88/NF-κB signaling pathway in the brain tissue	(80)

↑ indicates elevation or promotion, while ↓ means downregulation or inhibition. AD, Alzheimer's disease; Aβ, amyloid β; LRP1, recombinant low density lipoprotein receptor related protein 1; P-gp, P-glycoprotein; GFAP, glial fibrillary acidic protein; TNFα, tumor necrosis factor α; IL, interleukin; TLR4, toll-like receptor 4; IL, interleukin; MyD88, myeloid differentiation factor 88.

factor 88/NF-κB signaling pathway in the hippocampal tissue, thereby preventing the occurrence of PSD (80). Table II displays the relevant research progress regarding the use of saffron for treating NDs through its anti-inflammatory effects. Fig. 3 presents the anti-inflammatory mechanisms of saffron components in ND treatment.

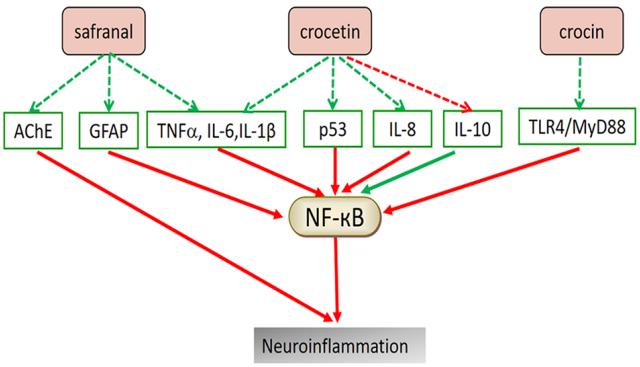

Figure 3. Potential mechanism of saffron compounds in treating neuroinflammation. The active components of saffron may inhibit the secretion of pro-inflammatory factors and increase the level of anti-inflammatory factors in serum, mainly through the NF-κB pathway. Red arrows indicate upregulation and green arrows indicate downregulation of the indicated pathway or factor. AChE, acetylcholinesterase; GFAP, glial fibrillary acidic protein; TNFα, tumor necrosis factor α; TLR4, toll-like receptor 4; IL, interleukin; MyD88, myeloid differentiation factor 88.

5. Improvement of mitochondrial dysfunction

Pathological process of mitochondrial dysfunction. Mitochondria are organelles with a double membrane structure found in the cytoplasm of eukaryotes containing extranuclear genetic material. Their internal membranes are the aggregation sites of respiratory chain complexes. Mitochondria, the main site of the body's energy metabolism, regulated the oxidative phosphorylation process and synthesize ATP, which may also be produced via glycolysis in microglial cells and astrocytes (81). Mitochondria generate energy and control the storage and release of Ca^{2+} to maintain the dynamic balance of the intracellular Ca^{2+} concentration. Furthermore, Ca^{2+} may participate in multiple cell activities, such as cell-matrix metabolism, cell apoptosis and initiation of signal transduction pathways (82). Mitochondrial dysfunction may prevent the aforementioned functions and is a major risk factor for neurodegeneration (83).

Treatment of PD with saffron. PD was the first ND associated with mitochondrial dysfunction. In the compact part of the substantia nigra, the nigrostriatal dopaminergic system and platelets of patients with PD, a 30% reduction in the activity of the mitochondrial respiratory chain complex I and a decrease in rate-limiting enzymes of the tricarboxylic acid cycle-ketoglutarate dehydrogenase complex were observed (84). In the rotenone-induced PD model in *Drosophila*, the level of mitochondrial enzyme activity in the *Drosophila* head was significantly reduced. Following treatment with saffron extract and CR, mitochondrial enzymes, succinate dehydrogenase and cytochrome *c* reductase returned to normal levels, indicating that saffron and its active constituents may improve mitochondrial dysfunction (68). Experiments using specific targeted small interfering RNA to knock down the expression of the C/EBP homologous protein (CHOP) revealed that CR-induced protection and inhibition of ER stress is mediated by inverting the 1-methyl-4-phenylpyridinium (ion)-induced decrease of Wnt protein through the CHOP pathway, thereby reducing cell damage and apoptosis, inhibiting mitochondrial dysfunction and maintaining ATP synthesis and $\Delta\psi m$ (85).

Treatment of CI with saffron. Transient CI is responsible for sudden, temporary and reversible neurological dysfunction. It has been demonstrated that mitochondrial dysfunction

Table III. Possible mechanisms of action of saffron in the treatment of neurodegenerative diseases by improving mitochondrial dysfunction.

Experimental animal	Model	Administration	Dose	Mechanism of action and target	(Refs.)
Drosophila	ROT-induced PD	Maintained on SME or CR-enriched medium	SME: 0.05%, 0.1%; CR: 0, 25 μM	Mitochondrial enzyme activity, succinate dehydrogenase and NADH-cytochrome *c* reductase activity returned to normal	(68)
PC12 cell	MPP^+-induced injury	Culture in medium containing crocin	0.1, 1, 10, 100 μM	CHOP expression ↓, Wnt ↑, caspase-3 activity ↓, LDH ↓, ATP synthesis ↑, cell cleavage ↓, cellular proteolysis ↓	(87)
SH-SY5Y cell	Oxygen-glucose deprivation	Culture in medium containing crocin	25, 50 mg/ml	$\Delta\psi m$, Opa1 expression ↑, Ca^{2+} concentration, Drp1 expression ↓	(88)
Rat	Middle cerebral artery occlusion induced CI	Duodenal injection	3 mg/kg	Opa1 expression ↑, Drp1 expression ↓, inhibition of astrocyte proliferation	(89)

↑ indicates elevation or promotion, while ↓ means downregulation or inhibition. PD, Parkinson's disease; ROT, rotenone; MPP+, 1-methyl-4-phenylpyridinium; CI, cerebral ischemia; SME, saffron methanolic extract; CHOP, C/EBP homologous protein; LDH, lactate dehydrogenase; Opa1, optic atrophy 1; Drp1, dynamin-related protein 1.

may occur after CI reperfusion (86). After pre-treatment of BI rats with CR, it was observed that CR increased the mitochondrial membrane fluidity, membrane phospholipid content, $\Delta\psi m$, mitochondrial respiratory function, respiratory enzyme activity and ATP content. CR also reduced MPTP opening and the free Ca^{2+} concentration and protected the hippocampal mitochondrial structure and function in rats with ischemic BI by significantly ameliorating the hippocampal mitochondrial pathology (87). In addition, CR can improve the energy metabolism of cells after oxygen-glucose deprivation, restore $\Delta\psi m$, reduce the intracellular Ca^{2+} concentration, upregulate optic atrophy 1 (Opa1) expression, downregulate dynamin-related protein 1 (Drp1) expression and restore the normal mitochondrial fusion and fission (88). Given the effects of the active constituents of saffron on improving mitochondrial dysfunction in cells, saffron extract was used to treat rats with focal brain ischemia/reperfusion injury. The results suggested that saffron extract significantly inhibited rat neuronal necrosis and astrocyte proliferation, upregulated the expression of Opa1, downregulated the expression of Drp1 and restored normal mitochondrial fusion and fission (89). Table III presents the relevant research progress of studies using saffron in treating NDs by improving mitochondrial dysfunction. Fig. 4 illustrates the mechanisms of saffron components in improving mitochondrial dysfunction for ND treatment.

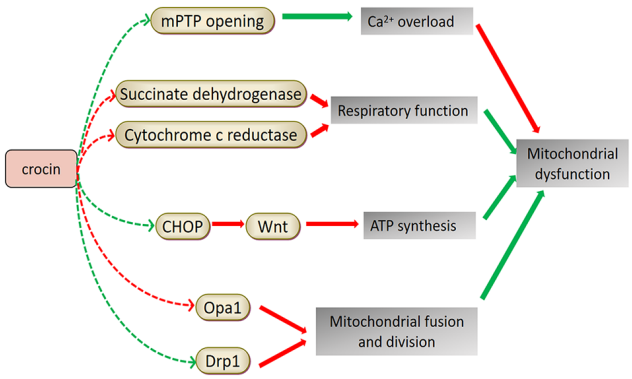

Figure 4. Potential mechanism of saffron compounds in improving mitochondrial dysfunction. Cell apoptosis and calcium overload may lead to mitochondrial dysfunction. Crocin is able to control calcium storage and release, prevent cell apoptosis, restore respiratory function and induce the synthesis of ATP, thereby protecting the mitochondria. It may also restore normal mitochondrial fusion and fission by regulating related proteins. Red arrows indicate upregulation and green arrows indicate downregulation of the indicated pathway or factor. mPTP, mitochondrial permeability transition pore; CHOP, C/EBP homologous protein; Opa1, optic atrophy 1; Drp1, dynamin-related protein 1.

6. Improvement of cognition and learning ability

Although the pathophysiological mechanisms remain to be fully elucidated, patients with AD frequently exhibit symptoms of reduced cognitive and memory functions, indicating that AD is closely related to memory impairment. Aβ deposition, synaptic loss, Tau phosphorylation and cholinergic system disorders are all possible factors responsible for neuronal damage (3,90). It has been reported that CR may increase the expression of brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) in the prefrontal cortex, thereby activating the BDNF-TrkB signaling pathway and increasing the expression of the memory-related protein postsynaptic density-95, which improves the learning and memory ability in AD rats (91). A bilateral frontal-cortex Aβ injection

Table IV. Possible mechanism of saffron in the treatment of neurodegenerative diseases by improving cognitive and learning ability.

Experimental animal	Model	Administration	Dose	Mechanism of action and target	(Refs.)
Rat	$A\beta_{1-40}$-induced AD	Microinjection of safranal	0.2 ml/kg	AChE ↓	(62)
Rat	$A\beta_{25-35}$-induced AD	Intracerebroventricular injection of crocin	40 mg/kg	Activation of the BDNF/TrkB signaling pathway, PSD-95, BDNF, TrkB expression ↑	(91)
Rat	$A\beta_{1-42}$-induced AD	Intraperitoneal injection of crocin	30 mg/kg	c-Fos expression ↓, neuronal apoptosis ↓	(92)
Rat	Ethidium bromide -induced MS	Microinjection of saffron extract	5, 10 μg	Amelioration of disturbances in oxidative stress parameters in the hippocampus	(109)

↑ indicates elevation or promotion, while ↓ means downregulation or inhibition. AD, Alzheimer's disease; AChE, acetylcholinesterase; MS, multiple sclerosis; AChE, acetylcholinesterase; BDNF, brain-derived neurotrophic factor; PSD-95, postsynaptic density-95.

trial in rats demonstrated that CR significantly reduced the number of TUNEL-positive cells in the cortical area 1 and decreased c-Fos expression in the hippocampus, thereby alleviating memory impairment due to Aβ deposition (92). Furthermore, electric shock experiments conducted in mice demonstrated that saffron extract prevented and improved the memory impairment of morphine-treated mice (93). Similarly, pentylenetetrazol-induced learning and memory deficits in rats were significantly alleviated by CR (94), although the mechanism of action requires further investigation.

Important neurotransmitters in the CNS, including dopamine, norepinephrine, acetylcholine and serotonin, act on the corresponding neurons and participate in short- and long-term memory (95). Acetylcholine-decomposing inhibitors of AChE are considered the primary treatment for AD because of their ability to improve cognitive impairment and the learning disabilities of AD. Studies have reported that the loss of cholinergic neurons in PD is higher than that in AD (96,97). Certain cholinergic fibers come from basal forebrain cholinergic neurons. Patients with non-dementia PD lose 30% of those neurons, whereas patients with PD dementia lose 54-70% (98). Saffron was found to be a source of novel AChE inhibitors for treating AD using *in vitro* enzymology and molecular docking methods (99). Furthermore, saffron extract and CR may act on muscarinic choline receptors to improve learning and memory ability (100,101). The accumulation and aggregation of lead (Pb) in the food chain may poison the nervous system. Experiments have demonstrated that Pb exposure may cause PD, resulting in memory and cognitive impairment, symptoms similar to dementia in AD (102,103). Saffron extract was able to improve Pb-induced dopamine and noradrenergic injuries by restoring tyrosine hydroxylase levels within the substantia nigra compacta, ventral tegmental area, locus coeruleus, dorsal striatum and medial forebrain bundle (104,105). Patients with MS usually suffer from cognitive impairment (106), with memory impairment and spatial perception disorders being the most common cognitive deficits (107,108). Saffron extract has a positive effect in improving learning and memory impairment and alleviate impaired hippocampal stress parameters in rats with ethidium bromide-induced MS (109). Table IV presents the relevant research progress on the use of saffron for treating NDs by improving cognition and learning ability. Fig. 5 illustrates the mechanisms of saffron components to improve cognition and learning ability for ND treatment.

7. Other effects of saffron and its active constituents

Relief of depression. Depression is one of the psychological symptoms of NDs. Studies have demonstrated that patients with NDs, including AD and PD, exhibit depressive symptoms. Furthermore, elevated levels of pro-inflammatory factors, such as IL-1β, IL-6 and TNFα, are frequently found in the cerebrospinal fluid of patients with depression, indicating the relationship between depression and the occurrence of neuroinflammation (110-115). The antidepressant mechanism of saffron has only been studied in the last decades. CR had a significant antidepressant effect in a chronic corticosterone-induced depression model in rats, as evidenced by a substantial reduction in IL-1β and SOD levels in the hippocampus, suggesting that the inhibition of inflammation and OS is associated with the antidepressant effect (116). Patients with depression usually have higher plasma corticosterone than normal individuals, it has been demonstrated that saffron water extract and CR were able to reduce the plasma levels of corticosterone in a rat model of depression (117,118). Increasing the transcriptional level of BDNF in the hippocampus may also have an antidepressant effect (119,120), demonstrating that saffron may alleviate the depressive symptoms of NDs. Therefore, saffron may not only relieve the main symptoms of NDs, such as nervous disorders and limb and cognitive dysfunction, but also have an antidepressant effect treating its potential complications, which is an advantage that other drugs do not possess.

Anti-epileptic effects. Epilepsy is a chronic disease characterized by sudden abnormal discharges of nerve cells in the brain, leading to temporary brain dysfunction. NDs are characterized by cell death and destruction of brain structures, which may

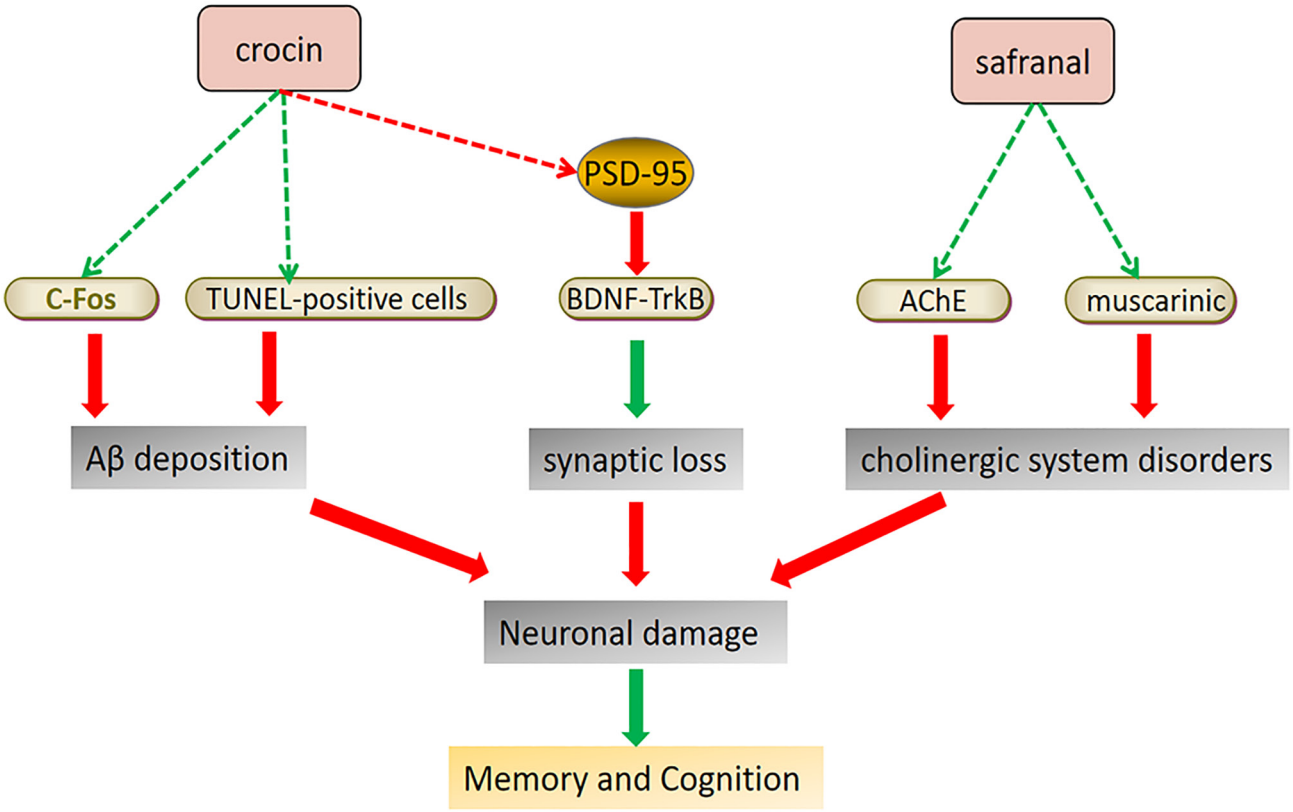

Figure 5. Potential mechanism of saffron compounds in protecting brain nerves and improving learning and cognitive ability. Neuronal damage to hippocampal tissue can affect cognition and learning ability. Saffron has a mitigating effect on the triggers of neuronal damage, such as Aβ deposition, synaptic loss and disorders of the cholinergic system. Red arrows indicate upregulation and green arrows indicate downregulation of the indicated pathway or factor. Aβ, amyloid β; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; AChE, acetylcholinesterase; PSD-95, postsynaptic density-95.

increase the risk of epileptic seizures (121). The appearance and increase in the levels of inflammatory cytokines, such as TNF, IL-1 and IL-6, is closely linked to the onset of epilepsy (122). CR and crocetin, the active constituents of saffron, inhibit the increase in the inflammatory cytokines to varying degrees (75). This finding suggests that the anti-epileptic action of saffron may be mediated by its anti-inflammatory properties.

Regulation of Ca^{2+} homeostasis. As the second messenger in neurons (123), Ca^{2+} regulates nerve synapses and has a vital role in cell growth, apoptosis, neuronal development and the regulation of various metabolic channels (124). An abnormal Ca^{2+} concentration may lead to cell damage and even apoptosis. The entry of Ca^{2+} from the external cell environment, a central characteristic associated with cell death, leads to Ca^{2+} imbalance and has an essential role in Ca^{2+} overload in NDs, such as PD and AD (125). For instance, the significant reduction of Ca^{2+} in the platelets of patients with early AD (detected as Aβ oligomer and associated with hypomnesia) increases the secretion of parathyroid hormone. This essential hormone maintains Ca^{2+} homeostasis and promotes calcium influx, leading to increased Ca^{2+} in brain cells, which causes dementia by damaging mitochondrial function and reducing cellular energy metabolism (126). Studies have reported that saffron and its active constituents may maintain Ca^{2+} homeostasis in neurons, but the specific mechanism of action remains to be elucidated (10,127). Therefore, the mechanism by which saffron regulates Ca^{2+} homeostasis requires further investigation.

8. Conclusions and prospects

Saffron has always been widely used for food coloring and flavoring. There has been a growing interest in using special diets with saffron, and scientists have been paying increasing attention to its safety while maintaining its taste (15-17). Furthermore, researchers have widely explored its nutritional quality and medicinal effect. Numerous phytochemicals in *Crocus sativus* have been proven to be the bioactive, therapeutic constituents of saffron. Saffron has been used in the clinical setting to treat cardiovascular and cerebrovascular diseases, mental disorders and abnormal blood lipid and glucose levels (128,129). Researchers have recently paid considerable attention to treating NDs, focusing mainly on diseases such as AD, PD, MS and CI (130). CR and crocetin, the active constituents of saffron with antioxidant effects, inhibit free radical formation and excitotoxic damage, thereby protecting neurons. These constituents may also reduce Aβ deposition, inhibit the abnormal aggregation of Tau protein, reduce the secretion of inflammatory factors, improve cognition and memory, improve mitochondrial dysfunction and regulate the homeostasis of metal ions in ND models. Furthermore, the pathways of the anti-inflammatory and antioxidant effects of saffron and its capacity to improve mitochondrial function and cognitive impairment are not independent; the interaction with one another may affect ND pathogenesis, thereby protecting nerve cells and preventing the further development of NDs. Fig. 6 illustrates the possible mechanisms of saffron for ND treatment.

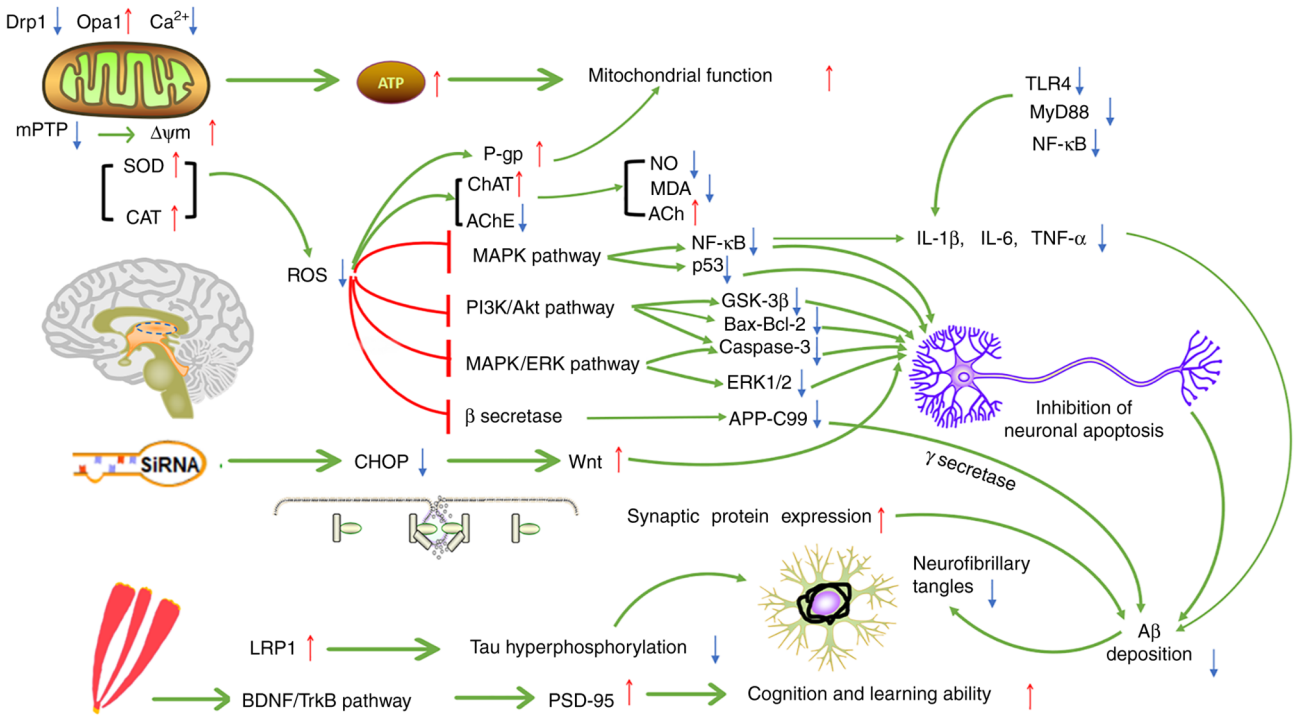

Figure 6. Possible mechanism of saffron in the treatment of ND. Through their antioxidant effects, the active constituents of saffron may reduce Aβ aggregation and the abnormal phosphorylation of Tau, thus alleviating the neurotoxicity caused by ROS. ROS may also lead to inflammation and damage to mitochondrial function. The active constituents of saffron may also act on different pathways to inhibit neuroinflammation and improve mitochondrial function. Different pathways may be connected and interact with each other, and may also represent the potential mechanisms of action of saffron in treating ND. T-type arrows indicate inhibition, pointed arrows indicate promotion, ↑ indicates upregulation and ↓ indicates downregulation. ND, neurodegenerative diseases; ROS, reactive oxygen species; siRNA, small inhibitory RNA; Aβ, amyloid β; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; AChE, acetylcholinesterase; LRP1, recombinant low density lipoprotein receptor-related protein 1; mPTP, mitochondrial permeability transition pore; SOD, superoxide dismutase; CHOP, C/EBP homologous protein; CI, cerebral ischemia; TLR4, toll-like receptor 4; MyD88, myeloid differentiation factor 88; MDA, malondialdehyde; NO, nitric oxide; CAT, catalase; ChAT, choline acetyltransferase; PSD-95, postsynaptic density-95; P-gp, P-glycoprotein; GSK3β, glycogen synthase kinase-3β; Opa1, optic atrophy 1; Drp1, dynamin-related protein 1; ERK, extracellular regulated protein kinase; TNFα, tumor necrosis factor α; IL, interleukin; MAPK, mitogen-activated protein kinase; Δψm, mitochondrial membrane potential; APP-C99, amyloid precursor protein C-terminal fragment 99.

The therapeutic potential of saffron has been proved in ND models. Its action on classic signaling pathways, such as NF-κB and MAPK, and its antioxidant and anti-inflammatory mechanisms, are also well known (65,76). Several beneficial pharmacological effects of saffron or its constituents have been confirmed in animal studies, but these effects have rarely been demonstrated in clinical trials. The results of a clinical trial suggested that saffron had similar effects to improve cognitive function of patients with AD as donepezil, while AChE levels were similar to those in the donepezil-treated group, indicating that saffron may inhibit the accumulation of senile plaques through its antioxidant effects, which is consistent with the results of a preclinical pharmacological study (131). Furthermore, the therapeutic effect of saffron on cerebral ischemia is similar to that of statins. Another clinical trial involving patients with AD demonstrated that oral saffron extract increased serum BDNF levels, inhibited inflammation and prevented neuronal apoptosis (132). Saffron has been clinically available to treat moderate AD, and antioxidant, anti-inflammatory and other mechanisms of its active compounds have been observed (133,134); it is thought that saffron has excellent potential for future application in treating NDs.

Furthermore, saffron has only been used as a traditional Chinese medicine to treat ND with limited efficacy. Its use in treating NDs is still experimental and further clinical studies are still required. The specific safety and effective dose for the human body are unknown and numerous factors are involved in determining its clinical efficacy. Accordingly, the following may be suggested: i) The use of saffron in the treatment of AD and CI may be investigated in subsequent clinical trials or drug developmental stages, whereas the pharmacological effect for treating other NDs requires further assessment in clinical trials; ii) saffron may be used to prevent the side effects (including anxiety, among others) of commonly used ND drugs, such as levodopa and memantine; and iii) clinical trials and safety evaluation should be conducted to assess the clinical value and effective dose of saffron and to elucidate the dose-response and dose-toxicity relationships of the active constituents of saffron.

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Availability of data and materials

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Authors' contributions

WY and XQ performed the literature search and wrote the first draft of the manuscript. QW, TZ, MZ and JP obtained funding, designed and conceived the study, supervised the preparation of the article and revised the manuscript. FC contributed to translation and data collection as part of the manuscript preparation. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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