Arch Iran Med. March 2021;24(3):233-252

doi 10.34172/aim.2021.35 http://www.aimjournal.ir

ARCHIVES OF IRANIAN MEDICINE

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Review Article

Prospects of Saffron and its Derivatives in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the most common form of dementia in the old age population, making it a worldwide concern. Unfortunately, few drugs have been presented for treatment of mild and moderate AD. To meet this need, more effective anti-AD agents are emerging. Accumulating evidence supports the beneficial roles of natural-based products in brain function, neurotransmission, neurogenesis, synaptogenesis, and the prevention of amyloid fibrillation and neuronal injury. Several *in vitro*, preclinical, and clinical studies suggest that saffron (its bioactive compounds) is a potential nutraceutical with antioxidant, radical scavenging, anti-inflammatory, hypolipidemic, hypotensive, neuroendocrine, and neuroprotective effects. It has also been proposed that saffron may delay the onset of AD, prevent its progression or help to attenuate the symptoms of the disease. Therefore, we performed a comprehensive search on this plant and its derivatives for AD treatment. Saffron and its active constituents interfere with AD by improving learning behavior, spatial memory, and cognitive function; protecting against neuronal loss; inhibiting beta-amyloid aggregation and neurotoxicity; preventing senile plaques and neurofibrillary tangle (NFT) formation; suppressing the acetylcholinesterase (AChE) activity; and reducing neuroinflammation. Given conclusive scientific findings, saffron and its derivatives might counter neurodegenerative diseases through multiple pathways. Further clinical trials are expected to confirm the neuroprotective properties of this herb and also to translate such findings to improve patients' outcomes.

Keywords: Acetylcholinesterase inhibitors, Amyloid beta, Apolipoprotein E, Neurofibrillary tangles, Saffron, Alzheimer's disease

Cite this article as: Zandi N, Pazoki B, Momeni Roudsari N, Lashgari N, Jamshidi V, Momtaz S, et al. Prospects of saffron and its derivatives in Alzheimer's disease. Arch Iran Med. 2021;24(3):233–252. doi: 10.34172/aim.2021.35.

Received: September 17, 2019, Accepted: October 4, 2020, ePublished: March 1, 2021

Introduction

Alzheimer's disease (AD) is the main etiology of memory loss, prompting irreversible progressive impairments in cognition and memory.[1] Both the incidence and prevalence of AD are increasing worldwide. It is estimated that in 2050, one out of 85 individuals will suffer from AD.[2] Thus far, several mechanisms have been proposed for AD induction or progression. The cholinergic hypothesis refers to reduction of acetylcholine (ACh) in the central cortex of the brain – an area that involves functional skills.[3] Hence, acetylcholinesterase (AChE) inhibitors like donepezil have been found effective in improving mild and moderate to severe AD symptoms.[3] The amyloid cascade hypothesis (ACH) suggests a lack of balance between production and clearance of amyloid-beta (Aβ), which leads to nerve cell dysfunction and death.[4] On the other hand, intracellular neurofibrillary tangles (NFTs) block neurotransmitters and cause neuronal cell death.[5] Tau oligomers are accumulated in β-sheet conformation and produce NFTs.[6] Also, high concentration of Aβ triggers NFT formation, and accumulation of NFTs in neurons lead to cell death.[7] Another hypothesis points to the fact that the ε4 and ε3 carriers of the apolipoprotein E gene (*APOE*) are more prone to AD; notwithstanding, the ε4 allele is the main genetic risk factor for late-onset AD. Apolipoprotein E (ApoE) is known to regulate lipid and protein homeostasis in the brain.[8-10] In addition to ApoE, several other genes have also been implicated in AD such as polymorphisms in sortilin related receptor 1 (SORL1), clusterin, complement component receptor 1 (CCR1), Cluster of differentiation 2 associated protein (CD2AP), Cluster of differentiation 33 (CD33), Ephrin type-A

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receptor 1 (EPHA1), and Membrane spanning 4-domains A6E (MS4A4/MS4A6E) genes.[11]

Oxidative stress and free radicals are effective factors for behavior and memory impairments in age-related neurodegenerative disease.[12] Genetic factors,[13] neuroinflammation,[14] type 2 diabetes, environmental factors, stroke, and diet[15,16] have also been proven to be involved in AD onset and/or progression, although aging is still the main risk factor for AD.[17]

Considering the shortcomings of current treatments for late-onset AD and regarding the positive impact of plant species in AD treatment (i.e. *Melissa officinalis, Nigella sativa, Boswellia* spp. and *Cinnamon* spp.) natural products are considered as high priorities for treatment of neurodegenerative diseases.[18] Saffron is one of the spices extracted from stigmas of the Persian herb *Crocus sativus* L. and is usually used in cooking[19] and contains four main constituents; safranal, crocin, crocetin, and picrocrocin.

Traditionally, saffron is used for gingival sedation, catarrhal healing, expectoration, improving appetite and digestion, nerve sedation and anticonvulsant, improving sweating, and as antispasmodic.[20,21] During the past two decades, various clinical and experimental studies have revealed that saffron and its bioactive constituents have therapeutic functions as anticonvulsant, anti-hypertensive, anti-spasmodic,[22] cardioprotective, anti-atherosclerotic,[23] anticancer,[24] antidiabetic,[25] antioxidant, antiparasitic,[26] anti-inflammatory, analgesic,[27] and immunomodulators.[28] They might be involved in modulation of smooth muscles,[29] gastrointestinal,[30] respiratory,[31] and reproductive[32] systems. Up to now, the majority of pharmacological experiments on saffron and/or its components have focused on their probable significant effects on CNS. The antidepressant,[33] anticonvulsant,[34] and antianxiety properties of saffron were reported, while it may improve memory impairments, tremor,[35] opioid withdrawal syndrome,[36] and was shown to have a broad spectrum of protective effects on the CNS. The stigmas, corms and phytochemicals of *Crocus sativus* could improve neuronal impairments,[37,38] such as Parkinson's disease, by reduction of dopamine in the substantia nigra,[39] suppression of neurotoxicity by diminishing oxidative damage,[40,41] repression of neuroinflammation due to increased intraocular pressure in order to avert retinal ganglion cell death in patients with glaucoma,[42] and improvement of neurodegenerative retinal diseases[43] and visual function in age-related macular degeneration patients.[44] *In vivo*, administration of saffron improved memory impairment induced by ethanol, aluminum (Al), morphine, ketamine, and arsenic.[41,45-48] Extracts of saffron stigma have been presented to have antioxidant and anti-amyloidogenic functions and also inhibited Aβ aggregation and deposition.[21,49]

Active Constituents of Saffron

Phytochemical analysis showed that saffron contains nearly 150 volatile and some nonvolatile compounds, of which only a few have already been identified. Apocarotenoid glycosides (i.e. crocin); picrocrocin; volatile oil (i.e. safranal); carotenoids; lycopene; alpha-, beta-, and gamma-carotene; fatty oil and starch are the main constituents of this plant.[50] Crocin, and crocetin belong to carotenoids, while picrocrocin and safranal are monoterpene aldehydes. Crocin is a glucosyl ester of crocetin and the compound responsible for the red color of *Crocus sativus*. However, picrocrocin, a glycoside of safranal, provides the unpleasant taste of *Crocus sativus*. Safranal is the main component of saffron and is associated with its aroma.[48] Crocin and safranal isomers have bioactive properties for better absorption in the intestinal lumen.[51-53] It was shown that saffron hydrolyzes to trans-crocetin by intestinal enzymes immediately after the entrance to the lumen and absorbed through the intestinal wall by passive diffusion.[54,55] Crocin is not absorbed orally, after a single dose or repeated doses, but its oral administration produces a higher level of crocetin in comparison with intravenous administration.[56] However, crocin is highly detected in the intestinal tract following oral administration. Crocin can hydrolyze to crocetin when used orally, then the absorbed crocetin is partially metabolized to mono- and di-glucuronide conjugates.[55,57] Crocetin's affinity for binding to albumin is low, which facilitates its transmission to different tissues and helps to cross the blood brain barrier via transcellular diffusion more easily.[54,57,58] According to a recent study in 2019, fast intestinal absorption of saffron extracts leads to a higher serum level of crocetin compared with intravenous administration.[59] The active components of saffron have been shown to possess antidepressant and antitumor effects, while they are able to neutralize free radicals and reduce inflammation.[20,21] Taken together, saffron might be a candidate for research on neurodegenerative diseases. Therefore, this review provides an overview of recently published clinical, preclinical, and experimental studies on therapeutic approaches using saffron and its derivatives for different aspects of AD.

Amyloid-β, a Key Molecule in AD

There are three distinct types of amyloid beta including very short oligomers, Aβ derived diffusible ligands, and protofibrils. Amyloid precursor protein (APP) is produced in the brain and is a major source of neurotoxic Aβ.[60] In detail, β-site APP cleaving enzyme 1 (BACE1), the main β-secretase in the brain, facilitates APP conversion to C_{99}.[61,62] Later, Aβ is generated from C_{99} by activity of γ-secretase. The γ-secretase function is regulated by presenilin 1 and 2 (PSEN1, 2), and any mutation in these proteins leads to excessive production of Aβ, initiating the early onset of AD.[63] In normal physiological states, there is an equilibrium between the production and clearance of Aβ in the brain,[64] and any disturbance in Aβ elimination or its overproduction will result in AD.[62] Interestingly,

low amounts of Aβ propitiously contribute to neural development[65] and can restrain lipoprotein oxidation in cerebrospinal fluid (CSF).[66] In addition, at low concentrations, Aβ was shown to have neuronal protective effects,[67] whereas high levels of Aβ lead to neuronal dysfunction by disrupting synaptic function and inducing neurotoxicity through free radical formation. Free radicals are accumulated in cerebral vessels, initiating a condition called cerebral amyloid angiopathy (CAA). CAA is a situation in which the amyloid proteins are placed through cerebral blood vessel walls,[68] which happens abundantly in AD.[64,69-71] Besides, accumulation of Aβ disrupts Ca homeostasis in cells and induces excitotoxicity.[72]

Deposition of Aβ also triggers an inflammatory condition through the nuclear factor kappa-light-chain-enhancer of activated B cell (Nf-κB) signaling pathway, and activation of microglial cells disrupts central nerves system (CNS) homeostasis in the chronic state.[73] The soluble form of Aβ is attributed to production of imperative proteins related to memory function (i.e. dendritic spines[74]). Soluble Aβ was also shown to have a significant role in AD induction and progression, and its levels rise in the brain,[75] blood, and the CSF[76] of AD patients.[77] Therefore, accumulation or formation of Aβ plaque is an assessment factor for AD diagnosis[67,78] however, there is no known relation between severity of disease and the insoluble form of Aβ or the plaque numbers.[78]

Tau and Neurofibrillary Tangles in AD

Tau is a protein involved in assembling of tubulin into microtubules[79,80] able to interact with cytoskeletal proteins actin and spectrin. In physiological conditions, neurons are responsible for tau production; nonetheless, in certain pathologic situations, it is also generated by glial cells. Typically, tau proteins are expressed in the CNS; however, the footprints of their mRNAs were also detected in other tissues.[81] It seems that tauopathy leads to neural death and NFT formation, which was also correlated with neuronal disturbance and severity of AD.[82,83] Hyperphosphorylated tau is the main reason behind its neurotoxic properties and also participates in NFT production as a core component.[84,85]

In addition, accumulation of tau is correlated with various degenerative disorders such as AD, progressive supranuclear palsy, argyrophilic grain disease, Pick's disease, Parkinson-dementia complex of Guam, and corticobasal degeneration.[86] Thus far, NFTs and Aβ are the most important components of AD pathology. Indeed, AD-type NFTs are mostly observed in the brain of old individuals even when there are no Aβ plaques. People with NFTs in the brain share comparable symptoms like AD. Regarding resemblance of Primary age related tauopathy (PART) and AD symptoms, by some definitions, PART is considered as a pre-AD factor or a subtype of AD.[87] Despite common features of PART and AD, it has been demonstrated that PART possesses limited effects on memory and cognition compared to AD.[88]

Apolipoprotein E and AD

APOE is a gene encoding ApoE with 299 amino acids, mainly existing in astrocytes. ApoE regulates lipid homeostasis by modulating lipid transport between different cells and by the action of ApoE receptors in the brain.[9] Liver and macrophages produce ApoE in peripheral tissue and it plays an important role in cholesterol metabolism. ApoE-4 is reported as a risk factor for various disorders such as atherosclerosis, coronary artery disease, peripheral artery disease, type 2 diabetes, and stroke; such diseases are also correlated with AD onset.[89-92]

There are three major alleles of the *APOE* gene including ε2, ε3, and ε4[10] displaying contradictory effects on AD likely due to the difference in amino acid residues 112 and 158.[91] People carrying ε4 are more prone to AD than those carrying ε3, by far, especially the ε4 homozygotes. In contrast, ε2 was shown to reduce AD risk.[93-95] According to various genome-wide studies, ε4 is the key genetic risk factor for AD.[96,97] Conversely, some studies refute this statement because some people carrying the ε4 allele never experience AD. However, they are susceptible to AD twenty times more than others.[98] It was shown that females and ApoE-4 positive individuals can weakly regulate the interaction between microglia cells and amyloid plaques, leading to greater risk of AD.[98] In addition, it was reported that aging and ε4 allele synergistically increase the risk of AD.[8,99]

The findings of human and animal studies demonstrated that ApoEs mediate APP and regulate Aβ aggregation and clearance (ε4> ε3> ε2) via triggering a non-canonical mitogen-activated protein kinase (MAPK) signaling pathway.[8,100-102] It was stated that absence of ApoE leads to elimination of fibrillar Aβ deposition in the *APOE* gene knockout mouse model.[103] ApoE-4 carriers have more senile plaques and experience CAA more frequently than non-carriers,[104-106] enhancing the risk of AD.[107] It was indicated that the presence of the ε4 allele exacerbated the consequences of sedentary lifestyle and aerobic exercise on cognition in individuals who carry ε4 in comparison with those not carrying this allele.[108-110] Smoking tobacco,[111-113] mild to moderate alcohol consumption,[114] and diets rich in high saturated fats[113] have also been shown to be responsible for higher risk of AD in ε4 carriers.

Anti-oxidant Effect of Saffron

The antioxidant properties of *Crocus sativus* and its constituents were associated with their activities against the oxidative enzymes; glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD),[23] catalase (CAT),[115] glutathione reductase (GR), and glutathione-S-transferase (GST).[116] Thus, saffron and its bioactive compounds can modulate oxidative stress in

cellular organelles and molecules, providing an effective mechanism against neurodegenerative disorders such as AD. It was demonstrated that stressed animals have higher amount of malondialdehyde (MDA), as well as higher activities of GR, GPx, and SOD enzymes in the brain, liver and kidneys, with lower total antioxidant capacity, compared with non-stressed animals.[117] In stressed groups, the corticosterone level was raised, confirming the point that glucocorticoids are involved in chronic-stress-induced oxidative damages, neuronal damage, and impairment of antioxidant defense.[118,119] Chronically elevated glucocorticoids caused neurogenesis blockade, hippocampal volume loss, and atrophy of dendrites in hippocampal CA3 pyramidal neurons.[120,121] Clinically, these changes lead to stress-mediated impairments in spatial learning and memory. Treatment with *Crocus sativus* extract and crocins improved such damages in the stressed group compared with the control group through enhancement of cellular antioxidant and detoxifying pathways.[122]

It was shown that the antioxidant components of saffron, such as crocins, crocetin, safranal, and flavonoids have synergic anti-oxidative effects, as the saffron extract is more efficient than each component alone.[123] Therefore, saffron and its bioactive compounds suppress oxidative and neuronal damages, and can thus alleviate cognitive deficits. Several studies demonstrated that streptozotocin (STZ) induces brain glucose deprivation and oxidative stress in animal models. Reduced cerebral glucose uptake and energy metabolism results in severe and progressive memory loss and poor learning ability due to deficiency in hippocampal choline acetyltransferase content.[12,124] Glucose hypo-metabolism and impaired insulin signaling were implicated in early onset and persistent complications in AD. The behavioral alterations of STZ-lesioned rats were attributed to increased MDA, as well as reduced GSH, total thiol, and GPx activity in the brain.[125]

Crocins were shown to improve cognitive performance, restore GPx activity, reduce lipid peroxidation and MDA pool, and replenish total thiol content in STZ-injected mice.[125] *Striatum* was chosen for injection due to the fact that it is particularly susceptible to oxidative stress damage due to increasing endogenous levels of antioxidants. Cerebral hypoperfusion leads to excessive reactive oxygen species (ROS) generation, which overwhelms the brain's antioxidant machinery, especially in the cortex and hippocampus.[126,127] *Crocus sativus* extract and crocins improved chronic cerebral hypoperfusion-induced cognitive impairments in mice by means of their anti-oxidative properties. In mice with cerebral ischemia-reperfusion injury, safranal treatment significantly restored the hippocampal antioxidant capacity and total-SH content.[128] Moreover, safranal elevated MDA levels in a dose-dependent style in the rat hippocampus in one animal study which was performed on male NMRI rats and transient global cerebral ischemia model was induced using the four-vessel-occlusion method for 20 min.[23] *In vitro* experiments on neuronally differentiated PC12 cells demonstrated that stress stimuli (i.e. serum/glucose deprivation, hypoxia), triggers cellular oxidative stress events like decline in intercellular levels of GSH and SOD activity.[23,128] Crocin treatment in PC12 cells attenuated lipid peroxidation and preserved neuron morphology. These effects were correlated to restoration of the activity and expression of SOD, GR, γ-glutamyl-cysteinyl synthase (γ-GCS), and the GSH pool. As mentioned, acrolein activated MAPK/ERK signaling pathway in rat cerebral cortex, as verified by phosphorylation of upstream kinases ERK1/2, c-JNK and p-38, resulting in reduced GSH and an enhancement of MDA content, Aβ deposition, and tau phosphorylation. Co-administration of crocin modulated MAPK signaling pathways, limited MDA pool, reduced Aβ level and tau phosphorylation, and therefore, prevented neuron apoptosis (Figure 1).[129,130]

Inhibition of AChE Activity and Saffron

It was proven that there is a significant correlation between cholinergic deficiency and cognitive impairments in AD pathogenesis, depending on ACh level in the brain.[131] Cholinergic pathways encompass the medial forebrain cholinergic nuclei and distribute to the hippocampus, amygdala, and neocortex. AChE hydrolyzes ACh to choline and the acetyl group. AChE inhibitors (AChEIs) prevent this breakdown in the brain.[132] However, increased ACh precursors such as choline and lecithin are not useful, but AChEIs have been found to be significantly effective in improving cognitive impairments. Tacrine, donepezil, and rivastigmine are approved AChEI drugs for AD treatment,[133-135] while there are many natural products that can act similarly.[135] Crocins have been shown to inhibit AChE by enhancing ACh levels in synapses and ameliorating cognitive symptoms.[128,136] In a 22-week, double-blind controlled trial, participants with mild to moderate AD randomly consumed either a 30 mg/d capsule of saffron or 10 mg/day of donepezil. Data showed the AChE ratio was comparable for both groups, demonstrating that saffron displayed the same therapeutic effect on cognitive function as donepezil. Besides, patients consuming saffron experienced less vomiting, slightly more dry mouth, and hypomania (Figure 1).[5]

Inhibition of Aβ Aggregation by Saffron

It was reported that trans-crocetin decreased Aβ42 aggregation *in vitro* and increased the level of a key Aβ42 degrading enzyme: the Aβ42-degrading lysosomal protease cathepsin B (CatB). These data indicate CatB involvement in the degradation pathway of Aβ42 in AD. Additionally, the compound modulated the intracellular level of CatB, suggesting a potential mechanism by which the degradation ability of Aβ42 could be retrieved. Studies

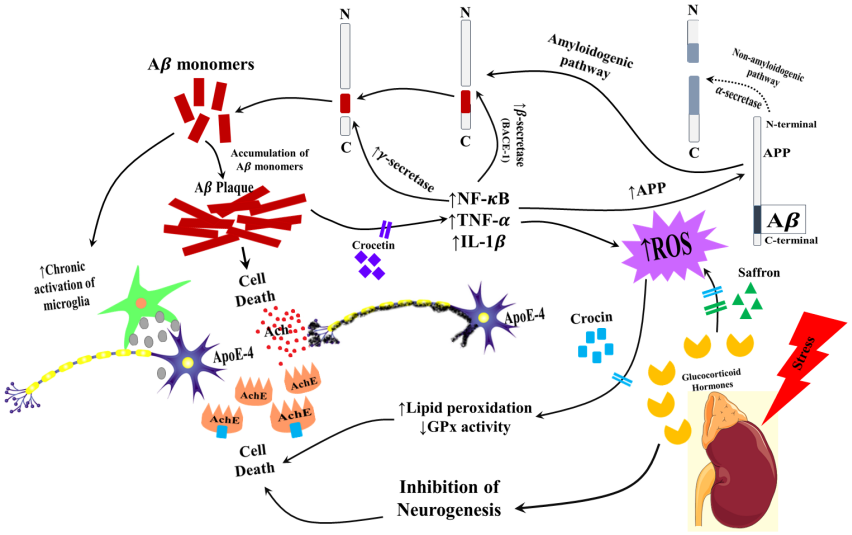

Figure 1. Inhibitory Effect of Saffron and/or its Derivatives in AD. Amyloid precursor protein (APP) is the main source of amyloid β plaques in the brain. Crocetin lowered the APP level by decreasing the inflammatory cytokines (IL1β, IL18, INFγ, and TNFα). Also, safranal and crocin inhibited the ROS formation resulted from increased stress. Crocin has been shown to inhibit acetylcholine esterase (AChE) and enhances the acetylcholine (ACh) in the neuronal synapses which ameliorates the cognitive symptoms.

also revealed that trans-crocetin has a positive effect on Aβ42 clearance and verified its neuroprotective effects on Aβ42-induced toxicity in hippocampal-derived cells, resulting in reduced cellular apoptosis.[7,137] However, it has been shown that crocetin affects multiple signaling pathways involved in neurodegenerative diseases such as extracellular signal-regulated kinase 1/2 (ERK-1/2) and caspases.[129,137] In the rat cerebral cortex, crocin alleviated tau phosphorylation by suppression of ERK and c-Jun N-terminal kinases (JNK) in acrolein induced oxidative stress and amyloid toxicity, showing that modulation of MAPK expression may be a mechanism underlying the crocin neuroprotective characteristic.[138] Supporting this notion, ERK was shown to mediate Aβ-induced tau phosphorylation.[139] In organotypic hippocampal slice cultures, both crocin and crocetin attenuated LPS-induced hippocampal cell death by decreasing nitric oxide (NO) release from activated microglia, highlighting their neuroprotection abilities. It was also demonstrated that crocin inhibited the Aβ42 formation and aggregation *in vitro*.[140,141] In the *in vitro* neuronal membrane bioreactor model, concomitant administration of crocin and Aβ-peptide repressed apoptosis and ROS production dose dependently.[142] In the *in vivo* model of AD, the compound inhibited Aβ-induced apoptosis through modulating the Bax/Bcl-2 ratio and cleaved caspase-3.[143] In the same manner, crocin prevented neuronal cell death caused by both internal and external apoptotic stimuli in tumor necrosis factor (TNF)-α treated pheochromocytoma PC12 cells via suppression of Bcl-Xs, LICE, and release of cytochrome c from mitochondria.[144] In another study, pretreatment with safranal reduced Aβ42 induced cell toxicity and apoptosis via MAPK and phosphoinositide 3-kinases (PI3K) pathways in PC12 cells.[137] In an Aβ-induced rat model, application of safranal (0.025, 0.1, and 0.2 mL/kg) for a week improved cognition deficits, and reduced CA1 neuronal loss and the hippocampal levels of MDA, ROS, protein carbonyl, interleukin 1β (IL-1β), IL-6, TNF-α, Nf-κB, apoptotic biomarkers and DNA fragmentation, glial fibrillary acidic protein (GFAP), myeloperoxidase (MPO), and AChE activity, while enhancing the SOD activity and mitochondrial membrane potential (Figure 1).[145]

Inhibition of Aβ-Induced Inflammation by Saffron

High concentrations of neuroinflammatory cytokines were observed in AD brains. Aggregation of Aβ plaques in the brain leads to enhanced neuroinflammatory cytokine levels such as IL-1β, IL-18, interferon-γ (IFN-γ), and TNF-α. This enhancement has been correlated with overproduction of APP in glial cells and upregulation of β- and γ- secretaseenzymes, which split APP and produce Aβ.[146,147] Animal studies pointed out that crocetin treatment lowered inflammation, prevented Aβ toxicity and reduced Aβ accumulation by enhancing tightness of the blood brain barrier (BBB), attenuating the increase of NF-κB p65 subunit and P53 in AD mice hippocampus. As a result, nitric oxide synthase (iNOS) production increased whereas proinflammatory cytokines such as IL-1β, IL-18, IFN-γ, and TNF-α diminished (Figure 1). [148]

Inhibition of tau Phosphorylation by Saffron

It was shown that corcin inhibited the beta-structure/random coil ratio of tau protein under fibril state and the aggregation of 1N/4R human tau protein in PC12 cells, which was correlated with its chemical structure. It was

proposed that carbonyl groups of crocin could interact with lysine residues of tau, leading to disruption of fibril formation.[149] As mentioned, in the rat cerebral cortex, crocin suppressed acrolein induced tau phosphorylation through modulation of ERK and JNK pathways.[138] Organophosphorus pesticides are accounted as important risk factors of AD. Mohammadzadeh et al reported that crocin (10, 20 and 40 mg/kg) improved spatial memory deficits in rats through inhibition of postsynaptic density protein 93 (PSD93) gene expression and tau phosphorylation. Besides, crocin significantly alleviated both oxidative and inflammatory parameters such as MDA, TNF-α and IL-6 levels, while increasing GSH in the hippocampus. The compound also reduced the plasma AChE activity and malathion-induced apoptosis in the hippocampus cells.[150]

Saffron and ApoE Related Approaches

Transcriptions of ApoE and ABCA1 are regulated via the linkage of peroxisome proliferator activated receptor γ (PPARγ) and liver X receptor (LXRs) to the retinoid X receptor (RXR).[151] It was shown that deletion of ABCA1 led to an increase in Aβ deposition in the murine brain, especially in ApoE-4 carriers, signifying its function in Aβ clearance.[152] ABCA1 regulates the ApoE lipidation by means of cholesterol efflux to ApoE and adjusts the ApoE level. On the other hand, binding of ApoE to Aβ changes the conformation of Aβ and increases its clearance.[153] In the Batarseh study, administration of saffron extract enhanced ABCA1 and PPARγ expression in murine brain, which led to Aβ degradation and deposition by modulating the BBB clearance and upregulation of ApoE-dependent Aβ clearance pathway (Figure 1).[148]

Clinical Trials on Saffron and AD

The mechanism of action of saffron in AD treatment and clinical trials is still under investigation. As previously mentioned, saffron showed similar efficacy as donepezil on patients with mild to moderate AD after 22 weeks by exploiting clinical assessment methods; the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) and Clinical Dementia Rating Scale-Sums of Boxes (CDR-SB).[154] Memantine is another approved drug for AD treatment, known to block the glutamatergic N-Methyl-D-aspartic acid (NMDA) receptors and their mediated excitotoxicity in the brain. Administration of saffron at low dose (30 mg/kg) resulted in the same outcomes as Memantine in moderate to severe AD patients. The Severe Cognitive Impairment Rating Scale (SCIRS) scores for both groups indicated no significant difference in the baseline and the final outcomes of the therapy.[155] Furthermore, the results of 16 weeks of saffron therapy versus placebo in individuals with mild to moderate AD were in line with the aforementioned clinical trials. Indeed, saffron significantly prevented cognitive impairments compared to placebo, highlighting the hypothesis that saffron is beneficial to people suffering from AD and memory deterioration.[156] Administration of saffron with standardized herbal medicine formula named sailuotong, containing *Panax ginseng* and *Gingko biloba,* showed potential effectiveness in improving working memory in comparison with placebo in healthy adults.[157] Concomitantly, application of saffron at a dose of 125 mg/d for a year enhanced cognitive function compared with the control group, suggesting that saffron may be an alternative medicine for AD drugs.[158] In a single blind randomized trial, 17 patients diagnosed with amnesic and multi domain mild cognitive impairment (aMCImd) were treated with saffron over a year. Neuropsychological assessment included a battery of psychometric tests assessing mood, activities of daily life, behavior, magnetic resonance imaging (MRI) 3T, and general cognitive function, while some patients were assessed via 256-channel electroencephalogram (HD-EEG). The findings of the study showed that saffron improved the MMSE scores, while amending the MRI, EEG, and event-related potential (ERP) in latency of P300 domain, suggesting that saffron may be a choice for MCI therapy (Table 1).[158]

In Vivo Interventions of Saffron and AD

Regarding the potential role of oxidative stress in pathogenesis of AD and other neurodegenerative diseases, the efficacy of saffron extract was investigated in BALB/c mice hippocampus cells with neuronal damage induced by D-galactose and sodium nitrite ($NaNO_2$). While D-galactose increased free radicals and $NaNO_2$ caused hypoxia, saffron inhibited the neurotoxicity resulting from their actions. This investigation suggested that in addition to anti-oxidative actions, saffron can also increase cerebral blood flow.[159] Administration of crocin in ddY mice after brain infarction induced by occlusion of a middle cerebral artery led to significant reduction of the infarcted area via passing the BBB. Interestingly, crocin was effective in a dose ten-fold less than α-tocopherol.[160] Similarly, 8 mg/kg crocetin reversed memory derangement in the vascular dementia model in rats including cortical and hippocampal hypoperfusion through permanent occlusion of common carotids, which has been confirmed in histopathological analysis.[161] In accordance, Hosseinzadeh et al[162] found that crocin (25 mg/kg) and saffron (250 mg/kg) attenuated memory deficits via decreasing oxidative stress in Wistar rats.

Zheng et al reported that following cerebral ischemia in C57BL/6J mice, pre-treatment with crocin and saffron inhibited oxidative stress parameters such as MDA and NO, while it enhanced the GPx, SOD, and iNOS activities. In addition, other oxidative markers, phosphorylation of ERK1/2, and the expression of membrane G protein-coupled receptor kinase 2 (GRK2) was reduced. The structure of cortical microvascular endothelial cells was

preserved by crocin.[163]

In a similar manner, pre-treatment with crocin and saffron in rats modulated the CAT and Na-K ATPase activities as well as aspartate and glutamate levels.[40] Increased lipid peroxidation is known as a marker of oxidative stress and IP exposure of saffron extract in Wistar rats led to lipid peroxidation reduction and amelioration of mitochondrial function in synaptosomal fractions, which were predisposed to the neurotoxin mitochondrial toxin 3-nitropropionic (3-NPA).[126] IP and intrahippocampal administration of crocin significantly improved the indicators of spatial memory. In Wistar rats, application of crocin reduced the Bax/Bcl-2 ratio and apoptosis, while the ratio of autophagy markers Beclin-1 and LC3-II/LC3-I remained unchanged.[143]

Moreover, administration of crocin improved sporadic AD induced by STZ in Wistar rats. According to the result of the Passive Avoidance Test and Maze Task Performance, memory and learning deficits were attenuated in the crocin group.[164-166] From a molecular viewpoint, crocin decreased MDA levels while elevating the total thiol level and the GPx activity in contrast to STZ.[165] Likewise, pretreatment with NCSe (combination of *Nardostachys jatamansi*, crocetin and selenium) in Wistar rats attenuated STZ-elicited oxidative stress by reducing thiobarbituric acid reactive substance level and increasing the glutathione, GPx, GST, and CAT activities, resulting in better performance in passive avoidance test and Morris water maze. Notably, this study mentioned that a multi-substance approach can be more potent than singular therapy.[167] Pretreatment of Wistar rats with saffron extract or crocin for 21 days before predisposition to chronic stress showed a significant neuroprotective effect on the hippocampus and an escalation in anti-oxidative stress markers,[168,169] as well as the mRNA expressions of CAT and SOD.[170] In another study, pre-treatment with a low dose of saffron prevented learning deficits induced by scopolamine in Wistar rats whereas post-treatment with saffron extract significantly retrieved data storage and recognition memory.[171,172] These data are against with findings by Zhang et al.[173]

It has been reported that saffron extract modified morphine-induced memory deficits in mice,[47] which is in line with the study by Haghighizad et al which indicated the efficacy of saffron extract on improving morphine-induced spatial learning and memory deficit in rats. Other investigations achieved the same results in ethanol-induced memory deficits in Std-ddY mice.[174]

Moreover, administration of 15-30 mg/kg crocin in Wistar rats reduced ketamine (non-competitive NMDA receptor antagonist) induced memory impairments using the novel object recognition task.[48] Saffron extract attenuated the acetaldehyde-induced inhibition of

Table 1. Saffron and its Derivatives; Clinical Interventions in AD (Human Study)

Study Design	Study Assessment	Intervention		Number of Patients		Treatment Duration	Outcomes	Adverse Effects	Ref.
		Case	Control	Case	Control				
Mild to moderate AD	MMSEADAS-cog, CDR-SB	SE (15 mg twice/day), oral	donepezil (5 mg twice/day)	n = 24	n = 23	22 weeks	Effective as donepezil	Dizziness, dry mouth, fatigue, hypomania, nausea (adverse effects were similar in both treatment & control groups, except vomiting)	154
Mild cognitive impairment	MMSE	SE (125 mg/d), oral	-	n = 17	n = 18	12 months	Improvement of cognitive dysfunction	-	158
Healthy adults	Cognitive test scores, oddball task -ERP	Sailuotong (*Panax ginseng, Ginkgo biloba & Crocus sativus*) (120 mg/d)	Placebo	n = 8	n = 8	1 week	Increase of Sailuotong in alphabetic working memory & visual working memory	-	157
Mild to moderate AD	MMSEADAS-cog, CDR-SB	SE (15 mg twice/day)	Placebo	n = 22	n = 20	16 weeks	Improvement of cognitive function	Dizziness, dry mouth, fatigue, hypomania, nausea (adverse effects were similar in both treatment & control groups)	156
Moderate to severe AD	MMSE	SE (30 mg/d)	Memantin (20 mg/d)	n = 30	n = 30	12 months	Effective as Memantin in reducing cognitive decline	Nausea, vomiting, dry mouth, fatigue, dizziness, confusion, agitation, sedation (adverse effects were similar in both treatment & control groups)	155

SE, saffron extract; MMSE, Mini-mental state examination; ADAS-cog, Alzheimer's disease assessment scale-cognitive subscale; CDR-SB, Clinical dementia rating scale-sums of boxes; ERP, event-related potential; MRI, magnetic resonance imaging.

hippocampal long-term potentiation in Wistar rats.[175]

For *in vivo* studies, one of the best proficient models of AD can be imitated by chronic administration of aluminum due to the same neurotoxic pathological changes in the brain.[176-179] Al accumulation in the brain leads to oxidative stress in the hippocampus and the cerebral cortex including lipid peroxidation, and deterioration of endogenous antioxidant enzymes, protein kinases, and Na^+-K^+ ATPase in the cell membrane.[180-182] It was shown that short-term co-administration of saffron extract with Al alleviated the oxidative stress markers and the monoamine oxidase activity; however, there was no effect on cognitive function and memory capacity in BALB/c mice.[41] Oral administration of 100 mg/kg saffron extract reversed the arsenic neurotoxicity while it promoted cognitive and memory functions. This was accompanied by decreasing glutamate and aspartate levels in cortical and hippocampal areas in Wistar rats.[46]

Various parts of the human body are affected by aging which ultimately results in dementia and progressive brain dysfunction. Oxidative stress in lipids, proteins and nucleic acids[183-185] along with poor performance of the cholinergic system due to reduced AChE activity in different parts of the cerebrum[49] and synaptic plasma membranes[186] is the basis for the main hypothesis for memory impairment in aged humans and rodents. In the study by Papandrou et al, crocetin decreased lipid peroxidation and caspase 3 activity in both adult and aged mice although the AChE activity was reduced in only adult BALB/c mice, emphasizing the greater role of oxidative stress in cognitive dysfunction compared to the cholinergic system (Table 2).[136]

In Vitro Interventions of Saffron and AD

In vitro administration of safranal, crocetin, and dimethylcrocetin inhibited ds and preserved the SOD activity which were exposed to serum/glucose deprivation. Moreover, crocin suppressed the caspase-8 (an initiator caspase) activity and increased the survival time of neuronal cells. It was indicated that the anti-oxidative ability of crocin was more than α-tocopherol (a form of vitamin E) at the same dosage.[188] Comparison of different saffron carotenoids revealed that 10 µM crocin is more potent than tricrocin and dicrocin in terms of reducing the GSH and caspase 3 activities in PC12 cells.[160]

Saffron and crocetin showed neuroprotective effects on H_2O_2 induced toxicity in human neuroblastoma SH-SY5Y cells by diminishing ROS products and caspase 3 activity.[136] Pretreatment of PC12 with 10-50 µg crocin in the neurotoxic state, induced by acrylamide, reinforced the neuroprotective effect of this compound. Indeed, crocin suppressed intracellular ROS production and apoptosis in these cells.[189] The same neuroprotective action of crocin was recorded in PC12 cells toxicated by either glucose or high levels of ROS.[190] In crocin pre-treated neurotoxic PC12 cells, the ratio of Bax/Bcl-2 decreased due to the apoptosis inhibitory effect of this compound.[189,191,192] Crocin downregulated TNF-α receptor activity in PC12 cells (mainly through the suppression of Bcl-2 mRNA expression) and increased caspase 3 activity. Besides, crocin prevented intracellular ROS formation elicited by daunorubicin.[144] In another study, 10 µM of crocin significantly restored ethanol induced NMDA receptor dysfunction and improved memory impairment in hippocampal slices of male Wistar rats.[45] Crocin suppressed 1-methyl-4- phenylpyridinium-induced endoplasmic reticulum stress and mitochondrial dysfunction in PC12 cells.[193] It is well established that microglial cells play pivotal roles in CNS homeostasis, but chronic activation of microglial cells predisposes neuronal cells to the inflammatory state by producing inflammatory cytokines including IL6, IL1β, TNF-α, and Nf-κB transcriptional activity as well as NO release. It was shown that saffron extract repressed the expressions of these elements in BV2 mouse brain microglial cells.[194] Overall, the neuroprotective features of crocin are mainly attributed to reduction of pro-inflammatory cytokines and neurotoxic factors (Table 3).[187,194]

Safety

Animal Studies

Considering the worldwide application of saffron, monitoring the probable adverse effects of this plant and its bioactive components seems necessary. Acute oral application of saffron in mice and rats was shown to be safe. Following IP administration in mice, the 50% lethal dose (LD_{50}) for saffron was reported as 1.6 g/kg, while for oral intake, LD_{50} was 4120 ± 556 mg/kg.[195] Administration of 3 g/kg crocin (IP and PO) for two days did not cause any mortality in mice; therefore, it was deduced that crocin is the safest substance of saffron.[196] Safranal exhibited LD_{50} values of 0.75 mL/kg and 3.5 mL/kg for IP and oral administration in male Wistar rats, respectively.[196] In rats, sub-acute IP exposure to saffron ethanolic extract decreased body weight, red blood cell (RBC) count, hemoglobin (Hb), and hematocrit (Hct). Conversely in a dose-dependent manner, white blood cells (WBC), alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzymes, serum urea, uric acid, and creatinine (Cr) levels increased. Pathological findings represented some mild to moderate liver and renal damage.[197]

Evaluation of saffron regarding spermatogenesis index in rats showed oral administration of 200 mg/kg saffron for 28 days reduced tubular differentiation index, spermatogenesis index, and repopulation index.[198] Another *in vivo* study demonstrated that crocin (90 mg/kg) for 21 days increased the low-density lipoprotein (LDL) level, while decreasing alkaline phosphatase (ALP) and albumin levels, without serious injuries in main organs even after

Table 2. Saffron and its derivatives; in vivo interventions in AD (Animal Study)

Animal Type	Disease Model (AD)	Intervention		Number of Animals		Treatment Duration	Outcomes	Adverse Effects	Ref.
		Case	Control	Case	Control				
BALB/c mice	D-galactose (90 & 120 mg/kg), i.p	SE (30 mg/kg/d), i.p	Water, i.p	1. amnestic treatment (n = 10) 2. amnestic prophylaxis (n = 10)	1. amnestic control (n = 10) 2. normal control (n = 10)	15 days	Improvement of learning memory impairment in amnestic induced groups	-	[159]
Wistar rats	3-NPA (20 mg/kg/d), i.p	SE (1 mg/kg/d), i.p	Saline, i.p	n = 6	n = 6	5 days	SE improves mitochondrial function via reduction in LP		[126]
Wistar rats	STZ, i.c.v	Crocin (15 & 30 mg/kg/d), i.p.	Vehicle	n = 15	n = 15	One day pre-surgery & continued for 3 weeks	↓ Memory deficits at higher dose	↑ Weight loss (crocin> crocin+STZ)	[164]
BALB/c mice	Aluminum chloride (50 mg/kg/d), oral	SE (60 mg/kg/d), i.p	Water, oral	n = 10	n = 10	5 weeks	No changes in cognitive function; ↓ MAO & AChE activity, ↓ LP & GSH		[41]
BALB/c mice	-	SE (60 mg/kg/d) i.p	Saline, i.p	Aged n = 8 Adult n = 8	Aged n = 8 Adult n = 8	7 days	Improvement of learning & memory impairment, ↓ LP, ↑ total brain antioxidant activity, ↓ caspase-3 activity in both aged and adult groups	-	[136]
Mice	Morphin (5 mg/kg), s.c	SE (50,150,450 mg/kg), i.p	Saline, i.p	n = 8	n = 8	3 days	Improvement of memory impairment	-	[47]
Wistar rats	Arsenic (100 mg/kg), oral	SE (100 mg/kg), gavage needle	-	n = 6	n = 6	-	Improvement of learning ability, ↓Glutamate & aspartate levels	-	[46]
Wistar rats	Amyloid β (100 ng/μL), i.p & i.h	Crocin (150, 300, 600 nm), i.p & i.h	-	-	-	-	Crocin ↑ Spatial memory, ↓ brain death		[143]
Wistar rats	Ketamine (3-25 mg/kg), i.p	Crocin (15, 30 & 50 mg/kg), i.p	Vehicle	n = 8	n = 8	3 days	Revision of memory deficits at 50 mg/kg	-	[48]
Wistar rats	STZ (3 mg/kg on day 1 & 3), i.c.v	Crocin (100 mg/kg), oral	Vehicle	-	-	21 days	Improvement of cognitive function, ↓ MDA, ↑ total thiol content & GPx activity		[165]
Wistar rats	STZ	Combination of Nardosatchys jatamansi extract (200 mg/kg), crocetin (25 mg/kg) & selenium (0.05 mg/kg), oral	Saline, oral	-	-	15 days	↓ Cognitive dysfunction		[167]

Table 2. Continued

Animal Type	Disease Model (AD)	Intervention		Number of Animals		Treatment Duration	Outcomes	Adverse Effects	Ref.
		Case	Control	Case	Control				
Wistar rats	Ethidium bromide (3 µL), i.h	SE (5–10 µg), i.h	Saline	*n* = 8	*n* = 8	1 week	Improvement of spatial learning & memory improvement, restoration of antioxidant status to the normal levels in hippocampus	-	212
Rats	Chronic cerebral hypoperfusion	crocetin (8 mg/kg), i.p	Control	-	-	-	Prevention of neuropathological alterations in hippocampus, improvement of spatial learning memory	-	161
Wistar rats	BeCl2 (86 mg/kg), oral	crocin (200 mg/kg), i.p	-	*n* = 8	*n* = 8	7 days	↓ Oxidative stress, ↑mRNA expression of SOD & catalase	-	170
Wistar rats	Scopolamine (0.2 mg/kg), i.p	crocins (15 and 30 mg/kg), i.p	-	*n* = 10	*n* = 10	2 days	Crocins (15 mg/kg) ↓ Memory impairment & ↑ recognition memory		171
Wistar rats	scopolamine (0.2 mg/kg), i.p	SE (250 mg/kg), oral	-	-	-	-	↓Hippocampal LTP	-	175
Std-ddY mice	Ethanol (10 ml/kg), po	Crocin (50 to 200 mg/kg), p.o	-	-	-	2 days	↓ Learning behavior impairments & memory retrieval deficits		174
C57BL/6J mice	Carotid occlusion-reperfusion	Crocin (5,10,20 mg/kg)	-	*n* = 10	*n* = 10	21 days	Neuroprotective effect, ↓ GRK2 translocation from the cytosol to the membrane, ↓ ERK1/2 phosphorylation, ↓ expression of MMP-9 in cortical microvessels	-	163
Std-ddY mice	Scopolamin (0.5 mg/kg), i.p & ethanol (10 mg/kg), oral	SE, oral	-	-	-	Single dose	↓ Memory impairment	-	173

Table 2. Continued

Animal Type	Disease Model (AD)	Intervention		Number of Animals		Treatment Duration	Outcomes	Adverse Effects	Ref.
		Case	Control	Case	Control				
Wistar rats	(60 mg/kg), STZ i.p	Crocin (7.5, 15, 30, 60 mg/kg), i.p	Saline, i.p	*n* = 6	*n* = 6	30 days	Improvement of learning & memory impairments		166
Wistar rats	Scopolamin (0.75 mg/kg), s.c	SE (10,30,60 mg/kg), i.p	Vehicle, i.p/s.c	*n* = 10	*n* = 10	-	↓ Memory impairment	-	172
Wistar rats	Chronic stress	SE (30 mg/kg) Crocin (15, 30 mg/kg, s.c	Vehicle s.c	*n* = 10	*n* = 10	21 days	Prevention of learning impairments & memory deficits; ↓ oxidative stress	-	169
Wistar rats	Cerebral ischemia	SE (50, 100, 250 mg/kg), i.p Crocin (5, 10, 25 mg/kg), i.p	Saline	*n* = 7	*n* = 14	-	Improvement of spatial cognitive abilities	-	**162**
Wistar rats	Chronic stress	SE or crocin (30 mg/kg), i.p	Saline, i.p	*n* = 6	*n* = 6er group	21 days	Prevention of brain oxidative damage, ↓ LP & MDA, ↑ GPx, SOD, GR	-	168
ddY mice	Middle cerebral artery obstruction	crocin (10 mg/kg), i.v.	-	-	-	1 day	↓ Infarcted area via passing BBB	-	160
Wistar rats	Amyloid β (5 μg/μL),	Safranal (0.025, 0.1, 0.2 ml/kg/day), p.o	-	*n* = 11	*n* = 11	1 week	↓ CA1 neuronal loss, the hippocampal MDA, ROS, protein carbonyl, interleukin 1β (IL-1β), IL-6, TNF-α, Nf-κB, apoptotic biomarkers & DNA fragmentation, glial fibrillary acidic protein (GFAP), myeloperoxidase (MPO), AChE activity, ↑ SOD & mitochondrial membrane potential	-	145
Wistar rats	Malathion (100 mg/kg/d), i.p	Crocin (10, 20, 40 mg/kg), i.v	Saline, i.p	*n* = 6	*n* = 6	14 days	↓ PSD93, tau phosphorylation, MDA, TNF-α, IL-6, plasma AChE activity & malathion-induced apoptosis in hippocampus cells, ↑ GSH	-	150

LPO, Lipid peroxidation; GSH, Gluthatione; AChE, Acetylcholine esterase; GPx, Gluthatione peroxidase; SOD, Superoxide dismutase; IL, Interleukin; MDA, Malondialdehyde; PSD93, Postsynaptic density protein 93; GFAP, Glial fibrillary acidic protein; TNF-α, Tumor necrosis factor; Nf-κB, Nuclear factor kappa-light-chain-enhancer of activated B cell (Nf-κB); BCR, Beryllium chloride; LTP, Long-term potentiation; GR, Gluthatione reductase; BBB, Blood brain barrier; GRK2, G protein-coupled receptor kinase 2; ERK1/2, Extracellular signal-regulated kinase1/2; i.p, Intraperitoneal; i.c.v, Intracerebroventricular; s.c, Subcutaneous; i.h, Intrahippocampal; p.o, per os
↓: decrease, ↑: increase

Table 3. Saffron and its Derivatives; *In Vitro* Interventions in AD

Ref.	Cell Type	Study Model of AD	Intervention		Number of Cells		Treatment Duration	Results
			Case	Control	Case	Control		
187	-	Kinetic analysis of AChE	Crocin, crocetin, dimethylcrocetin, safranal	Control & galanthamine	-	-	-	AChE inhibition activity; Safranal> crocetin > dimethylcrocetin
49	-	Ferric-reducing antioxidant power & Trolox-equivalent antioxidant capacity	SE (50:50 water & methanol)	-	-	-	-	↑ Cognitive function via antioxidant & antiamyloidogenic activity, ↑ cognitive function
45	Hippocampal slices of male Wistar rats	Ethanol	Crocin (10 μM)	-	-	-	-	Revision of the inhibitory effect of ethanol on NMDA receptor-mediated responses
193	PC12	1-methyl-4-phenylpyridinium (MPP+)	Crocin	-	-	-	-	↓ MPP+-induced ER stress & cell injury
189	PC12	acrylamide (5 mM)	Crocin (10–50 mM)	-	-	-	-	↓ Apoptosis, ↓ Intracellular ROS formation
194	BV2 mouse microglial cells; hippocampal slice cultures organotypic of rats	LPS	Crocin, crocetin	-	-	-	-	↓ Cell death, ↑ anti-oxidative & anti-inflammatory effects
160	PC12	Serum-free & hypoxic induced cell-death	Crocin (10 μM)	-	-	-	-	↓ infarcted areas
188	PC12	Serum/glucose deprivation	Crocin (10 mM)	-	-	-	-	↓ LP content, ↑ SOD activity, protected neuron's morphology
136	Neuroblastoma SH-SYS5 human cells	H2O2	SE & crocetin (1-125 μmol)	-	-	-	-	↓Cell death, ↓ caspase 3 activity & ROS formation
144	PC12	TNF-α & daunorubicin	Crocin (1–10 μM)	-	-	-	-	↓ Cell death, ↓ both internal & external apoptotic stimuli
190	PC12	High glucose (4.5, 13.5, & 27 mg/mL)	SE (5 & 25 μg/mL), crocin (10, 50 μM)	-	-	-	4 days	↓ Cell death, ↓ ROS production & glucose toxicity
141	-	Na_2HPO_4 NaCl (100 mM)	Crocin (15.4 μM)	-	-	-	-	↓ Amyloid fibril content of Aβ; inhibits Aβ aggregation
140	-	Na_2HPO_4 NaCl (100 mM)	Crocin (15 μg/mL)	-	-	-	-	↓ Aβ40 average fibril length, ↓ formation of Aβ fibril formation
213	PC12	-	Crocin (10 μg/mL)	-	-	-	-	↓ Tau protein fibrillation

SE, Saffron extract; MPP+, 1-methyl-4-phenylpyridinium; LPS, Lipopolysaccharide; LP, Lipid peroxidation; SOD, Superoxide dismutase; ROS, Reactive oxygen species
↓: decrease, ↑: increase

exposure to 180 mg/kg of crocin.[196] This was in line with results of a study by Taheri et al since IP administration of crocin at concentrations of 50, 100 and 200 mg/kg once a week for four weeks caused no elevation in Cr, ALT, AST, ALP, uric acid and urea levels in rats. Pathological examination revealed no significant hepatic toxicity.[199]

Three weeks of safranal oral administration (0.1, 0.25, 0.5 mL/kg) in rats, led to reduction of triglyceride, cholesterol, ALP, RBC count, platelet count, Hb, and Hct, while the level of blood urea nitrogen (BUN) increased. However, no pathological lesion in organs (liver, spleen and heart) or toxicity effect on the cellular and humoral immune system were detected.[200] Oral administration of 4000 and 5000 mg/kg saffron in BALB/c mice for 5 weeks demonstrated that sub-chronic exposure to saffron decreased the RBC and WBC counts and increased BUN and Cr, indicating renal dysfunction.[201] Worth mentioning, usually in animal studies, saffron is used at high doses although it exhibited protective effects in lower doses.[202]

Human Studies

Like other plant extracts, several side effects were reported for saffron such as nausea, vomiting, anxiety, headache, dizziness, epistaxis, bloody diarrhea, and numbness. It was assumed that at doses of 12-20 g, saffron can be fatal.[203]

In a clinical study on healthy volunteers, standing systolic blood pressure and mean arterial pressure were reduced by receiving saffron tablets (400 mg); however, there was no change at a dose of 200 mg.[204] In another study, hematologic factors and the coagulation system were not disturbed by saffron tablets (200 and 400 mg).[205] Safety of crocin was investigated in a double-blind, placebo-controlled trial in which healthy volunteers received crocin tablets (20 mg) for a month. Crocin tablets decreased amylase, partial thromboplastin time, and the WBC count, demonstrating that crocin was relatively safe.[206] Pregnant women with fetuses at gestational ages between the first and twentieth weeks were susceptible to abortion if they received saffron at high doses.[207] In addition, uterine contractions induced by saffron have been suggested as a mechanism for abortion.[204,208,209] At the beginning of the active phase of labor, administration of saffron capsules (250 mg) reduced mean anxiety score and mean fatigue score,[210] while saffron capsules in the active phase of labor reduced pain. The infant and mother did not show any toxicity in the saffron group compared with controls.[211]

Based on the findings of animal studies (LD_{50} values), crocin might be the safest component of saffron, and no significant damage has been mentioned for this compound at pharmacological dosage. At high concentrations, saffron and its constituents showed some developmental toxicity on animal infants. Exposure to high levels of saffron was shown to increase miscarriage rates in pregnant women, suggesting avoidance of high doses during pregnancy.[202]

Conclusion and Future Perspectives

Statistics confirm that AD remains a global growing health concern. A wide range of natural and synthetic molecules have been studied for their ability to either prevent or counteract AD initiation, progression, and complications. The findings of this study indicate that saffron and/or its components target various regulatory molecules involved in AD. Regarding its pleotropic effects on the nervous system, including anti-amyloid, anti-AChE, anti-inflammatory, and anti-oxidant features, along with its inhibitory effect on tau hyper-phosphorylation, and upregulation of ApoE activity, it seems that saffron could find its niche in AD therapy with substantial potential as a therapeutic nutraceutical with the advantage of low toxicity and easy accessibility. Further studies, particularly clinical trials, are now required to determine whether saffron and its bioactive phytochemicals may be suitable for AD or other neurodegenerative disorders. Other clinical trials are warranted to examine the safety and efficacy of various doses of the plant and improved formulations with better pharmacokinetics and bioavailability are needed. Several reports have raised questions about the safety and efficacy of saffron or its derivatives, especially at high doses, whereas some studies have shown no adverse effects. It is suggested that the mode of administration and the duration of saffron therapy are also critical factors that can significantly affect the efficacy of AD treatment. Since saffron is a part of daily diets in many Asian countries and seems non-toxic, it is obligatory to investigate whether dietary supplementation with saffron may be a beneficial preventive or slowing nutritional strategy for neurological disorders.[212]

Authors' Contribution

NZ, BP, NMR and NAL: collection and/or assembly of data and interpretation, manuscript writing; SM, VJ, AHA and SA: provision of study material, conception and design, and final approval of manuscript. All the authors have read and approved the manuscript.

Conflict of Interest Disclosures

None.

Ethical Statement

Not applicable.

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