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Association of *Crocus sativus* with Cognitive Dysfunctions and Alzheimer's Disease: A Systematic Review

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Abstract: Alzheimer's disease (AD) is known as a principle basis of cognitive impairment in the elderly population. Current available therapeutic approaches are not applicable enough for the reason of intolerable side effects, low patients' compliance due to the short half-life, and biopharmaceutical limitations. Therefore discovery alternative and multi-targeted therapeutic approaches are sought. Crocus sativus is a distinguished medicinal plant with a wide range of biological and pharmacological belongings, exclusively antioxidant and anti-inflammatory possessions. Following PRISMA guidelines, a systematic review was conducted by search in Electronic databases comprising Scopus, PubMed, Web of Science, and Embase using the keywords cognitive OR cognition OR memory OR nootropic OR Alzheimer OR amnesia OR dementia AND "Crocus sativus" OR saffron OR crocin OR crocetin OR safranal until 21st February 2020. The results display that saffron and its major constituents are capable of having remarkable properties on memory and cognitive deficiency. Cell signaling pathways, anti-oxidative stress experiments, modulation in inflammatory and proinflammatory mediators, tauopathy, clearance of amyloid-beta aggregation, and histopathological alterations are indicated. Cognition tests and scores allied with memory loss are specified. The administration of saffron in experimental models seems to be an encouraging attitude in AD even though it is recommended that further studies for the valuation of pharmacokinetic properties and bioavailability enhancing carriers of these phytochemicals must be directed.

Keywords: Alzheimer's disease; amnesia; cognitive disorders; crocetin; crocin; dementia; memory impairment; nootropic; saffron; safranal.

1. Introduction

Neurodegenerative diseases, described by irreversible neuron loss and gliosis (nonspecific changes of glial cells), including disorders such as Parkinson's disease (PD), frontotemporal degeneration (FTD) and Alzheimer's disease (AD) [1]. AD is postulated through the process of getting old and the most prevalent reason for dementia in aged people. AD shows, unlike clinical manifestations; however, cognitive dysfunctions and memory loss are the main hallmarks that can lead to subsequent incapability and death [2]. AD recruits with diminishing in memory, and according to its progression, AD can be classified into three phases: a. the preclinical phase, there is not any modification in cognitive ability, b. the mild cognitive impairment (MCI) phase, and c. dementia phase [3]. The global annual expenses of

dementia were assessed (US) $818 billion in 2015. Besides, AD may inflict passionate stresses on patients and their relatives. It has been appraised that 115.4 million individuals will suffer from AD until the year 2050 [3,4].

1.1. Pathogenesis of Alzheimer's Disease.

The hippocampus is an anatomical construction in the brain medial temporal lobe that is imperative for learning and memory. It consists of four subregions, which consist of the dentate gyrus (DG), hippocampus proper (CA1, CA2, and CA3), subicular complex, and entorhinal cortex (EC). A short-term high-frequency train of inducements to major synaptic pathways raises the amplitude of the excitatory postsynaptic potentials in the target hippocampal neurons. This enablement is entitled long-term potentiation (LTP), which is substantial in the duty of memory deficiencies. Abundant convoluted mechanisms anticipated in the pathogenesis of AD [5]. There is a low chance (5–10%) for the incidence of genetic history. AD is associated with mutations in three different genes: presenilin-1 (PS1), presenilin-2 (PS2), and amyloid-β precursor protein (APP-β) [6-10]. Aggregation of β-amyloid protein (Aβ) in neuritic plaques (NPs) and hyper-phosphorylation of tau (τ) protein (tauopathy) in neurofibrillary tangles (NFTs) are the most momentous pathophysiological changes [11-14]. The formation of Aβ deposits leads to an immune response with the contribution of astrocytes and microglia [5,11]. Reactive microglia can produce free radicals, which can be fatal for neurons. Activation of some T-cells has been observed in brain parenchyma of AD sufferings. Such cells release inflammatory mediators, including; interleukin (IL)-1, IL6, Ɣ-interferon, and tumor necrosis factor-alpha (TNF-α) .It has approved that usage of non-steroidal anti-inflammatory drugs (NSAIDs) modulates the inflammatory factors responsible in AD through inhibition of cyclo-oxygenase (COX). Evidence-based studies are endorsed effects of oxidative stress on augmentation of formation and accumulation Aβ and hyper-phosphorylation of tau. It was observed that alteration in levels of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and total antioxidant status (TAS) in erythrocyte and the plasma could be considered as a marker for pathological distinctions in the brain of patients with AD [15]. Environmental factors and apolipoprotein-E are claimed as other probable causes of AD [16,17].

1.2. Therapeutic Strategies in Alzheimer's Disease.

As a reason for the intervention of multiple factors in the initiation of AD, a multi-target-directed ligand strategy will be an effective therapeutic approach [18]. There are two groups of FDA-approved medications for the management of AD symptoms. These drugs act through two mechanistic pathways; Non-competitive N-methyl-D-aspartate (NMDA) receptor antagonists (memantine) and cholinesterase inhibitors (ChEIs) (donepezil, galantamine, and rivastigmine) [19,20]. These chemicals, synthetic therapeutic agents, have some side effects, short half-life, and fluctuated bioavailability, which leads to narrow indications [18]. There is a growing demand for the usage of phytochemicals, nutraceuticals, and phytochemical components for the control and management of numerous diseases such as AD. Phosphatidylserine, Ginko, huperzine, and vinpocetine are obtainable natural-based products in the market, which can ameliorate mental functions in patients who have dementia. By considering the tendency to discover novel drugs and the opulent therapeutic potential of

phytochemicals, investigation of the anti-AD or memory-enhancing effects of those would be well-intentioned [16,21-24].

1.3. Phytochemical Properties of Saffron.

Saffron is botanically named as *Crocus sativus* L. (Iridaceae) has three major components; crocins, (the source of coloring pigment), the glycoside picrocrocin (a precursor of safranal and contributed its explicit flavor), and safranal (the deglycosylated form of picrocrocin and the major organoleptic principle of the stigmas) [25,26]. Crocin, crocetin are carotenoids of saffron, and safranal is a monoterpene aldehyde [27,28]. Carotenoids are bioactive components with great antioxidant potential [29]. The high-performance liquid chromatography (HPLC), ionization-mass spectrometry (APCI-MS), (Fourier transform near-infrared) FT-NIR, and ultraviolet-visible (UV-Vis) spectrometry analytical procedures are chiefly applied for qualitative and quantitative analysis of saffron components [30,31].

1.4. Biological and Pharmaceutical Aspects of Saffron.

Numerous pharmacological effects have been endorsed to saffron and its components including; anticancer [32,33], neuroprotective (anti-Alzheimer's disease, anti-Parkinson, anticonvulsant, antidepressant, anxiolytic, and anti-schizophrenia) [34-37], anti-ischemic (heart, muscular, kidney, and brain ischemia,) [38], antioxidant [39], antinociceptive, antitussive, hypolipidemic, anti-diabetic [40], antidote (against snake venom, acrylamide, etc.), anti-obesity and anorectic, Aphrodisiac, nephroprotective [41], anti-hypertensive, anti-genotoxic, cardioprotective [42], antimicrobial, hepatoprotective, gastroprotective [43] and anti-inflammatory effects [44-46]. Saffron is wildly used in food flavoring and coloring, anti-pruritic and emollient, cosmetics, textile dye, perfume, and pharmaceutical industry [47,48].

2. Study Design

Herein we conducted the systematic review coincident to the established PRISMA guidelines [49]. A literature search was targeted on the electronic databases of Scopus, PubMed, Embase, and Web of Science. Other databases were searched to avoid missing related articles. There was no time limitation for the searched articles. The search was accomplished by using the following search strings in the title/abstract/keywords: cognitive OR cognition OR memory OR nootropic OR Alzheimer OR amnesia OR dementia AND "*Crocus sativus*" OR saffron OR crocin OR crocetin OR safranal. Reclaimed articles were imported to EndNoteX6 reference management software. All articles were separately screened for, duplicity, and eligibility by authors individually.

2.1. Inclusion Criteria.

Articles were included with the following properties; I) original research articles, II) articles published in English, III) use of any form of *Crocus sativus* and its major constituents (crocin, safranal, and crocetin), IV) researches with sufficient outcomes associated with objectives of the study and V) published and in press articles before 21st February 2020.

2.2. Exclusion Criteria.

Articles were excluded with the following properties; I) the study did not evaluate AD, cognitive disorders, and any related conditions of memory loss, II) no related experimental assays were used III) *Crocus sativus* or its constituents were used as a positive control or as bio-enhancers IV) only abstract was available V) the study directly claimed that crocin was originated from *gardenia*.

2.3. Data Extraction.

In total, the search yielded 451 articles of those 92 met inclusion criteria (Figure 1). In total, 60 studies were based on animal-based experiments, 29 studies were based on cellular or in vitro studies, and 8 studies were based on human clinical trials. Some of the selected articles had in vitro and in vivo studies both. In vivo studies containing nonspecific experiments were not categorized as a separate result. Studies evaluating AD, other cognitive incapacities due to neuronal disturbances, and examining healthy cognition performance were included.

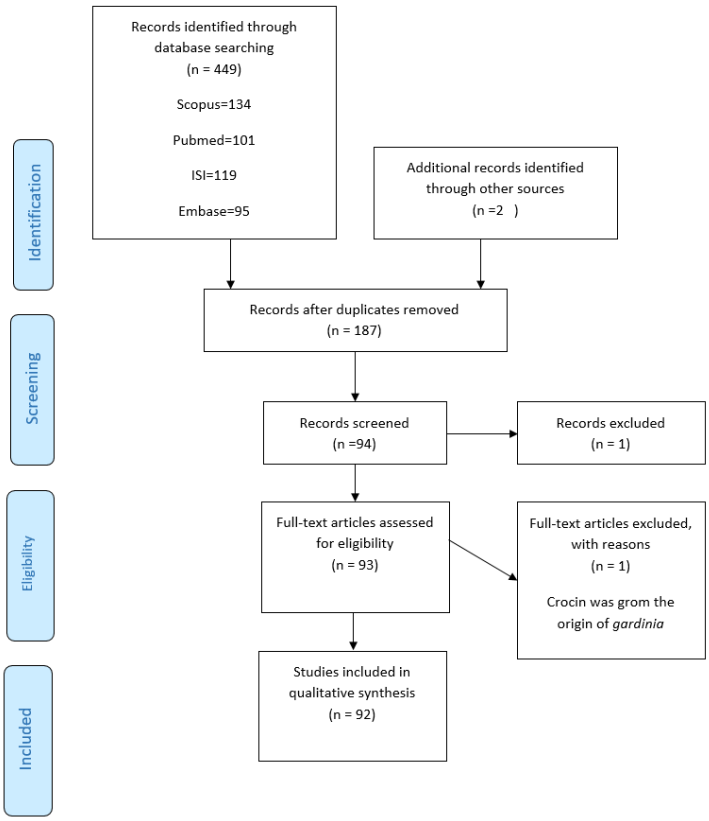

Figure 1. Study Selection Diagram.

3. Mechanisms Involved in Memory Enhancing and anti-Alzheimer's Disease Potential of *Crocus sativus*

This is the first systematic review for evaluation of the association between consumption of saffron and its therapeutic approach in Alzheimer management and cognitive dysfunctions. Characterized extracted data of in vivo, in vitro, and clinical studies are collected in (Table 1-3)**,** respectively.

In animal-based studies, some cognition measuring experiments were performed, and associated results show memory enhancement. Open Field Test (OFT) has indicated an increase in a. the number of square crosses, b. the number of rearings, c. time spent in the center

and number of center entries.Y-Maze Test has demonstrated an elevation in a. spontaneous alterations (%) and b. the number of arm entries. Passive Avoidance Test (PAT) has shown a. increase in Step-Through Latency (STL) and b. the decrease in Initial Latency (IL). Morris Water Maze (MWM) is used for examination of spatial learning and memory, which has indicated a. attenuation in Escape Latency (EL), b. increase in Time Spent in Target Quadrant (TSTQ), and c. increase in swimming speed. Radical 8-Arm Maze (RAM) has demonstrated a reduction in working memory errors and reference memory errors. Novel Object Recognition Test (NORT) is shown elevation in a. Discrimination Index (DI) and b. Exploration time of the novel object. Other memories assessing behavioral experiments such as; Novel Object Location Test (NOLT) Elevated Plus Maze (EPM), Barnes Maze, T-Maze, and Sucrose Performance Test were also used (142) [50-53]. Improvement of the cognitive dysfunctions or memory impairments through pretreatment with saffron and its constituents is demonstrated apparently in preclinical studies [45].

Clinical trials, *in vivo* and *in vitro*, showed that Aβ acts a main function in the pathogenesis of AD. Aβ accumulation is responsible for senile plaques formation, activation of oxidative damage, neuronal inflammation, apoptotic death, and cognitive impairments. Mitigating the ROS formation via the consumption of saffron, safranal, crocin, and crocetin is one of the significant mechanisms in the AD therapeutic approach. Increase SOD, CAT, GSH, oxidized glutathione (GSSG), and TAC and alleviation of Malondialdehyde (MDA), nitric oxide (NO), Lactate dehydrogenase (LDH), carbonyl proteins and DNA damage are desired (143) [54-56]. Obtaining free radical scavenging capacity is probable through 2,2,1-diphenyl-1-picrylhydrazyl (DPPH), Thiobarbituric acid reactive substances (TBARS) and ferric reducing antioxidant power (FRAP), as well. Attenuation of the β-cleavage of APP may lead to protection combat AD (144, 145). The balance of pro- and anti-apoptotic Bcl-2 family proteins (e.g., Bcl-2 and Bcl-w versus Bad, Bim, and Bax) plays a pivotal function in apoptosis neuronal cells. Thus expression levels of these proteins are seemingly changed in the vulnerable neurons in AD [57].

Exposure to Aβ peptide initiates the activation of astrocytes and microglia leads to proinflammatory chemokines and cytokines such as IL-8, IL-6, IL-1β, and TNF-α [58]. Elevation in IL-1β level is accompanied by over activation of c-jun-N-terminal kinases (JNK), caspase-3, and mitogen-activated protein kinase (MAPK),; all of these contributed to apoptosis and synapse loss. IL-1β also attenuates the release of acetylcholine in synaptic space, which contributes to further cognitive impairments in AD. The release of chemokines can induce the migration of monocytes from the peripheral circulation into the brain and initiates inflammatory responses (146), whereas pretreatment with saffron attenuated its activations and regulation of p38 MAPK, and AKT pathways [59]. Lipocalin-2 (LCN2) is a principle molecule that acts in cell viability, inflammation, and many other biological reactions. LCN2 manipulations may be a potential therapeutic target in CNS inflammatory conditions for instance, AD [60]. Activation of the 78-kDa glucose-regulated protein (GRP78) of the endoplasmic reticulum may participate in phosphatidylinositol 3-kinase (PI3K)/AKT pathway of management the neurological disorders [61]. Transcription factor nuclear factor-kappa B (NF-κB) is a crucial regulator of innate immunity. NF-κB may regulate AD pathophysiologically caused by apolipoprotein-E, metabolic syndrome, APP, ROS production, and environmental factors [62]. Neurotrophin BDNF plays the role of regulating diversified neuronal structure and function in the development of the adult CNS and has known as one of the most significant signaling molecules for the development of the nervous system. It has been

reported that BDNF plays a critical role in neuronal survival, synaptic plasticity, and memory [63]. Cyclic AMP (cAMP)-responsive element-binding protein (CREB) is a nuclear transcription factor that contributed to AD [64].

Aβ consists of 36-43 amino acids and is a natural product of sequential cleavage of an integral membrane protein, the amyloid precursor protein (APP). APP can undergo cleavage in one of two non-amyloidogenic and amyloidogenic pathways. In the first pathway, cleavage by the enzyme α-secretase prevents Aβ formation. It leads to the production of the sAPPα fragment that has some beneficial properties such as promoting neuronal growth and neuroprotective effects. However, the sequential cleavage by γ-secretase(containing presenilin 1 at its catalytic core) and β-secretase, a beta-site amyloid precursor protein-cleaving enzyme 1 (BACE-1) lead to the formation of Aβ. Then Aβ releases into the extracellular spaces. Mediation APP and BACE-1 are considered as effective pathways in the mitigation of memory impairment by saffron [65]. Inhibition of activator and signal transducer of transcription 3 (STAT3) is a molecular mechanistic target combat AD and cognitive dysfunctions [66].

Consequently, JAK/STAT3 cascade and Glial Fibrillary Acidic Protein (GFAP) run as a dominant controller in astrocyte goings-on [67]. Postsynaptic density protein 93 (PSD-93) and PSD-95 are significant scaffolding proteins in the regulation of Aβ-persuaded synaptic dysfunctions [68]. Neutralization caspases activities or expression are considered as therapeutic targets combat neurodegenerative disorders like AD [69]. Peripheral blood mononuclear cells are considered as Aβ plaque suppressors through modulating BBB penetration by chemokines such as CXCL-10 [70]. CHOP gene (C/EBP-homologous protein) is a pro-apoptotic transcriptional factor that may cause AD in vivo, then deactivating this gene will be an option for the discovery of novel drugs with anti-AD properties [71]. It has been demonstrated by augmentation in levels of corticosterone risk of AD induction increased through Aβ plaque formation [72]. Matrix metalloproteinases (MMPs) are responsible for neuroinflammation in the pathogenesis of AD, MMP-2, MMP-3, and MMP-9 are the most important ones that are correlated with Aβ plaque formation [73]. An extracellular signal-regulated kinase (ERK) plays a functional role in restoring neuronal damages [74]. Insulin-like growth factor 1 (IGF-1) may play a pivotal role in the progression and pathogenesis of AD [75]. The p53 protein has appeared to intermediate programmed cell death in neurons of AD by regulating levels of Bcl2 family proteins [76].

TAU hyperphosphorylation can alter various synaptic proteins via inhibition of PP2A and activation of GSK-3β. Therefore synaptic proteins and their levels of expression may cause an anti-inflammatory effect in the hippocampus of AD [77]. Mammalian target of rapamycin (mTOR) may correlate in Aβ plaque formation and tauopathy induced neurodegeneration [78]. Machine learning and pattern recognition techniques are appropriate for diagnosing AD and MCI from individual MRI scans. Another application of such methods is to predict clinical scores from individual scans. Relevance vector regression (RVR), the Mini-Mental State Examination (MMSE), Dementia Rating Scale (DRS), and Auditory Verbal Learning Test (AVLT) are the basic methods. Prediction and actual clinical scores are evaluated by Alzheimer's Disease Assessment Scale-Cognitive (ADAS-cog), MMSE, and DRS tests. This result underscores their utility for screening and tracking disease. In clinical practice, we visualize the usage of RVR to help practitioners diagnose and predict clinical outcomes. Individual MMSE items, especially those with some type of episodic memory referent, were the best predictors of incident cases of AD. Moreover, MMSE items displayed fluctuation, especially in the incidence of AD (147).

Table 1. Results of *in vivo* studies.

Animal/ Sample size (No. of groups)	Model of Study	Targeted compound /Duration/ Dosage/ Route of Administration	Cognition Test	Major Outcomes	Ref.
Rat/ 42, 6	malathion	Crocin/14 days/10, 20 and 40 mg/kg/ i.p.	MWM	↓EL, ↑TSTQ, ↑swimming distance, AChE activity=>NSD, ↓MDA, ↑GSH, ↓TNF-α, ↓ IL-6, ↓ PSD93 protein level, ↓TAU protein level at Thr205 and Ser404, phosphorylated and total GSK-3β=> NSD, ↓PP2A at the pY307-PP2AC protein level, ↓TAU mRNA expression, ↑PP2A mRNA level, ↓Bax/Bcl2 ratio, caspases (3, 8 and 9)=>inhibited	[77]
Mouse/ 56, 3	C57BL/6 J	Trans-crocin-4 (TC4)/50 , 150 mg/kg/ i.p.	N.M.	25 metabolites have been interpreted; 1) 3 of them had a potential role in neuroprotection closely related to AD 2) 5 of them contributed in the pathway of the steroid (Estrogen-Receptor beta (ERβ), 11-deoxycorticosterone (21-hydroxyprogesterone), corticosterone (11β,21-hydroxyprogesterone) and other corticosteroids) biosynthesis, even though 2 of them might be considered as neuroprotective agents	[79]
Rat/ 46, 4	Lipocalin-2	Saffron in a combination/ 28 days/ 16.5, 33 mg/kg p.o.	MWM	↓Neurological score after 2 h, ↓Neurological score after 24 h, ↓Brain Damage after Surgery, ↓number and size of infarcts, nerve cell degeneration, and Necrosis in the infarcts=>Were not observed, necrotic substances=>were absorbed, ↓EL, ↑TSTQ, ↑platform crossings, ↓IL-1α, ↓IL-12, ↓CXCL10, ↓LCN2 secretion, ↓p-STAT3 and p-JAK2 staining in astrocytes, ↓OGD-Induced Injury in Astrocytes	[80]
Mouse/ 60	AlCl3	Crocin/ 4 weeks 5 or 20 mg/kg p.o.	MWM, OFT	↑ the circulation around the periphery, ↓time to locate the platform, ↑serum levels of Aβ1-42, ↓cerebral cortex levels of Aβ1-42, deposition of Aβ1-42 in the hippocampal area=>suppressed, pathological changes of the levels of Ach, AchE and ChAT in the serum, cerebral cortex, and hypothalamus of the AD mice=>restored, ↑SOD, ↑GSH-Px levels in the serum, cerebral cortex, and hypothalamus	[81]
Rat/ 37, 5	6-OHDA	Crocin alone or accompanied by treadmill exercise/ 100 mg/kg/ i.p.	PAT, EPM	total net No. of rotations↓, STL↑, transfer latency↓, TNF- α levels in the striatum↓, TBARS levels in the hippocampus↓, total thiol concentration↑	[82]
Rat/ 56, 4	Trimethyltin chloride (TMT)	Crocin/ 30 days/ 25, 50 mg/kg/ i.p.	N.M.	↓Bax, ↓Caspase-9 levels, ↑Bcl-2, Pt and Aβ40 levels↓, BDNF levels↑, ↓IL-6, ↓IL-1β, ↓TNF-α, ↑the neuronal density of CA1, CA2, and CA3	[83]
Rat/ 30, 4	TMT	Crocin alone or accompanied by	N.M.	IGF1, Glycogen↑	[84]

Animal/ Sample size (No. of groups)	Model of Study	Targeted compound /Duration/ Dosage/ Route of Administration	Cognition Test	Major Outcomes	Ref.
		treadmill exercise/ 8 weeks/ 25mg/kg			
Rat/ 60, 5	Methamphetamine	Crocin/ 21 days/10, 20, 40, and 80 mg/kg/ i.p.	MWM	↓EL,↑ TSTQ, swimming speed=>NSD, ↑GSH, ↓GSSG levels, ↓MDA, ↑SOD, ↑GSH-Px, ↑GR, ↓IL-1β, ↓TNF-α, ↑Bcl-2 expression, ↓Bax protein expression, ↑BDNF, and CREB protein expression	[85]
Rat/ 48, 4	Aβ25–35	crocin 14 days/ 40 mg/kg/ i.p.	Y-maze, sucrose preference test	↓Attempting times, ↑correct reaction rate, ↑sucrose preference, ↓number of apoptotic cells,↑ Bcl-2, ↓Bax, ↓Caspase-3, ↓CHOP, ↓GRP78, ↓endoplasmic reticulum stress in hippocampus and PFC	[86]
Rat/ 70, 7	methylphenidate	Crocin/ 21 days/ 10, 20, 40, and 80 mg/kg/ i.p.	MWM, OFT	↓EL ↓ traveled distance, swimming speed=>NSD, ↑TSTQ, ↑ frequency of central square entries, ↑time spent in the central region, ↑ambulation distance and rearing number, ↑ GSH content, ↓GSSG, ↑SOD, ↑GSH-Px, and ↑GR, ↓MDA, ↓IL-1β, ↓ TNF-α, ↑Bcl-2,↓ Bax protein expression, ↑protein expression in BDNF and CREB total and phosphorylated forms	[87]
Rat/ 66, 6	Aβ1–40	Safranal/ 7 days/ 0.025, 0.1, or 0.2 ml/kg/ p.o.	Y-maze, NORT, RAM, PAT	reduction of alternation score=> prevented, ↑discrimination ratio, ↑STL, IL=>NSD, ↓working memory errors, ↓reference memory errors, ↓MDA, ↓Nitrite, ↓protein carbonyl, ↓ROS, ↑CAT, ↑SOD, ↑GSH, ↓IL-1β, ↓IL-6, ↓TNFα, ↓NF-kB, ↓caspase 3 activity, ↓DNA fragmentation, ↓GFAP in the hippocampus, ↓AChE activity, ↑MMP, ↓number of CA1 neurons	[88]
Rat/ 48, 5	TMT	Crocin alone or accompanied by treadmill exercise/ 8 weeks/ 25 mg/kg/ i.p.	EPM	↓weight levels,↑% Open Arm Entry,↑%Elapsed Time in the Open Arm, ↑Aerobic Power	[89]
Rat/ 48, 6	Post-traumatic stress disorder	Saffron and Crocin/ 28 days/ 10 µg/ ICV	Barnes Maze	↑corticosterone concentration, ↓Freezing Behavior, Search Strategies (direct, random and serial)=>NSD, ↓ No. of Errors in finding the target hole, ↓Traveled Distance, Latency Time=>NSD	[90]
Rat/ 36, 6	Hyoscine	Crocin/ 5 days/ 10, 20, and 40 mg/kg/ i.p.	MWM	↓EL, ↑TSTQ, swimming speed=>NSD, NMDA and AMPA protein expression =>NSD, CaMKII, pCaMKII, and ERK proteins=>NSD, a decrease of pERK protein level=>inhibited, ERK, CaMKII, NMDA and AMPA RNA expression=> NSD	[91]
Rat/ 30, 5	hypoxia	Crocin/ 3 days/ 25, 50, 100 mg/kg/ i.m.	MWM	↓EL, ↑TSTQ, ↑No. of crossings, ↑mitochondria of nerve cells, mitochondria swelling and cristae blurring=>improved,↑SIRT1 and PGC-1α proteins distribution, ↑the hippocampal density of SIRT1 and PGC-1α	[92]

Animal/ Sample size (No. of groups)	Model of Study	Targeted compound /Duration/ Dosage/ Route of Administration	Cognition Test	Major Outcomes	Ref.
Mouse/ 26, 3	APPsw	Crocetin/ 6 months/ 10, 30 mg/kg/ p.o.	MWM, NORT	↓ insoluble Aβs in the hippocampus, the cerebral cortex, and the cerebellum, ↓ EL, ↑TSTQ, ↑ time spent exploring the novel object, ↓ NF-κB-p65, ↓ p53 levels, ↓Aβ plaques, ↓TNF-α, ↓IL-1β, ↓IL-8, ↓IL-6, ↑ IL-10 levels	[93]
Rat/ 40, 5	morphine	Crocin/ 21 days/ 12.5, 25 mg/kg/ i.p.	N.M.	↓glucose level, serum cholesterol=>NSD, serum triglyceride level=>NSD, ↑BDNF and CREB gene expression in the VTA area, ↑serum BDNF level	[94]
Rat/ 32, 4	normal	Crocins alone and in combination with memantine/ 5, 15, and 30 mg/kg/ i.p.	NORT	↑Discrimination (D) index, Total exploration times=>NSD, Total motor activity=>NSD	[95]
Rat/ 40, 5	isolation stress	Crocin/ 21 days/ 30 and 60 mg/kg/ i.p.	NOLT, NORT	OLT; ↑Total time of object exploration, ↑novel object exploration time, ↑ Values of the main discrimination index (D2), ↑values of auxiliary discrimination index (D1) / NORT; ↑Total time of object exploration, ↑novel object exploration time, ↑ Values of the main discrimination index (D2), ↑values of auxiliary discrimination index (D1), serum corticosterone levels	[96]
Rat/ 40, 5	Aβ peptide (1-42)	Crocin/12 days/ 30mg/kg/ i.p.	Barnes maze, PAT	↓latency time to achieve the target hole, ↓number of errors to find the target hole, ↑ STL, ↓c-Fos, ↓number of TUNEL-positive cells in the CA1 region, cell death=>prevented, ↑PS amplitude, ↓fEPSP slope, LTP induction=>prevented	[97]
Rat/ 88, 8	streptozotocin (STZ)	Safranal alone and in combinnation with metformin/ 37 days/ 0.025, 0.1 and 0.4 mg/kg/ i.p.	MWM	↓hyperglycemia,↓ EL, ↑TSTQ, ↓distance swam, hippocampal neuron loss=> recovered, ↓MDA, ↓TNF-α, ↓Caspase-3,↑ SOD	[98]
Rat/ 48, 6	Chronic unpredictable Stress	Crocin/ 21 days/ 30 mg/kg/ i.p.	PAT	↑STL, ↑ IL to enter the darkroom, total dark compartment stay time=>NSD	[99]
Mouse, rat	5XFAD, STZ, scopolamine	IIIM-141 trans-4-GG-crocin (36 % w/w)/ 28 days/ 12.5, 25, 50, 100 mg/kg/ p.o.	MWM (STZ-rat), PAT (Scopopolamine-mouse)	MWM; ↓transfer latency time, ↓% change in transfer latency PAT; ↑transfer latency time, ↑ % change in transfer latency	[100]
Rat/ 35, 5	Tramadol	Crocin/ 28 day/ 30 mg/kg/ p.o.	MWM, PAT	TSTQ=>NSD, ↑traveled distance, ↑time delay for entering the dark compartment, ↓total time spent in the dark compartment, No. of entries to the dark compartment=>NSD, ↑total time spent in the light compartment, ↓No. of TUNEL positive cells in CA1, CA3 and DG of the hippocampus, DNS No. per unit area in CA3 and DG of the hippocampus	[101]
Rat/ NORT; 60/ NOLT; 48	Apomorphine	Crocins/ 3 days/ 15 and 30 mg/kg/i.p.	NOLT, NORT	NORT; ↑index D, exploration times=>NSD, NOLT; D index=>NSD, exploration	[102]

Animal/ Sample size (No. of groups)	Model of Study	Targeted compound /Duration/ Dosage/ Route of Administration	Cognition Test	Major Outcomes	Ref.
				times=>NSD, Total motor activity=>NSD	
Mouse/ 40, 5	pentylenetetrazol	Crocin/ 36 days/ 5, 10 and 20 mg/kg/p.o.	T-maze, NORT	↓Seizure severity score, ↑(%)spontaneous alternation, ↑(%)preference index, ↑ discrimination ratio, ↑SOD, ↓number of Nissl-stained dark neurons, ↓ NF-ΚB expression, ↓p-NF-ΚB expression	[103]
Mouse/ 32, 4	aflatoxin B1	Saffron tea/ 2 weeks 90 mg styles/200 mL (3.6 mg saffron/ mouse)/ p.o.	PAT	body weight gain, food and liquid intake=>NSD, ↑STL, IL=>NSD, ↓SS- and DS-AChE activity in whole brain and cerebellum, ↑ the activity of whole-brain DS-BuChE was at control levels, ↓liver SS-AChE and BuChE activity, ↓liver DS-BuChE, ↓MAO-A and MAO-B, ↓MDA in the whole brain, cerebellum, and liver, ↓GSH values in brain, cerebellum, and liver	[104]
Rat/ 30, 6	D-galactose	Crocin/56 days/ 7.5, 15, 30 mg/kg/i.p.	MWM	↓EL, ↑TSTQ, swimming speeds=>NSD, ↓MDA, ↓CML expression, ↑phosphorylation of Akt protein, MAPK activity through the elevation of pErk/Erk ratio↑, ↓NF-κB p65 protein level in the hippocampus, ↓ IL-1β and TNFα formations	[105]
Rat/ 40, 5	Chronic Restraint Stress	Crocin/ 21 days/ 30 and 60 mg/kg/ i.p.	PAT	↑STL, ↑ IL, ↓corticosterone levels in the hippocampus and frontal cortex	[106]
Rat/ 60, 6	Aβ(25-35)	Saffron / 21 days/ 5, 10 mg/kg/ i.p.	The Y-maze task, RAM	In vitro antioxidant; ↑DPPH scavenging, ABTS•+ scavenging (general experimental tests)/ In vivo; ↑short working memory, ↑(%)spontaneous alternation, ↑ No. of arms entries, ↓ Working memory errors, ↓reference memory errors	[107]
Rat/ 36, 6	hyoscine	Crocin/ 5 days/ 10, 20 , 40 mg/kg/ i.p.	MWM	↑TSTQ, Swim Speed in the Target Quadrant and Total Swim Speed=>NSD, ↓Latency Time to Find Platform, ↑ BDNF, ↑ p-CREB, ↑CREB	[108]
Mouse/ 18, 2	5XFAD	1 month/ Saffron; 50 mg/kg /Crocin; 10 mg/kg/ p.o.	N.M.	↓total Aβ and Aβo levels in the brain, ↓levels of monomeric Aβ40 and Aβ42 in the brain,↑ LRP1 and P-gp expressions, expression of the Aβ degrading enzyme NEP in brain homogenates↑, expression of IDE levels=>NSD, ↑ABCA1 expression, ↑ PPARγ expression, ↓IgG extravasation, ↑ claudin-5 expression in brain microvessels, ↑PSD-95 expression, ↑SNAP-25 expression, ↓ brain levels of IL-1β, ↓GFAP optical intensity	[109]
Rat/ 35, 5	streptozotocin	Crocin/ 6 weeks/ 15, 30 and 60 mg/kg/ i.p.	MWM	↓serum glucose levels, ↓EL, ↓traveled distance, ↑TSTQ, ↓TBARS level in the cortex, total thiol concentration in the cortex=>NSD	[110]

Animal/ Sample size (No. of groups)	Model of Study	Targeted compound /Duration/ Dosage/ Route of Administration	Cognition Test	Major Outcomes	Ref.
Rat/ 60	ketamine	Crocin/2, 5 or 10 mg/kg/ i.p.	PAT	STL=>NSD, the performance of the rats in the shuttle box=>improved	[111]
Rat/ 32, 4	6-OHDA	Crocin/ 6 weeks/30 and 60 mg/kg/ i.p.	PAT	↓TBARS levels in the hippocampus,↓ nitrite levels in the hippocampus, total thiol concentrations in the hippocampus and cortex=>NSD, GSH-Px=>NSD, ↑STL	[112]
Rat/ 10, 2	aging	safranal /30 days/ 0.5 mg/kg /i.p.	N.M.	↓MDA, ↑GSH-Px,↑ SOD, ↑ GST	[113]
Rat/ 24, 4	acrolein	Crocin/ 2 weeks/ 12.5, 25, 50 mg/kg/day/ i.p.	N.M.	↓MDA,↑ GSH-Px, ↓Aβ1–42,↓T231 phosphorylation state, ↓phosphorylation of Ser396, ↓p-Akt, levels phosphorylated and total GSK-3β at Ser9 GSK-3β=>NSD, ↓p-ERK1/2, ↓p-JNK, level of p-p38=>NSD	[114]
Rat/ 40, 5	ethidium bromide	Saffron/1 week/ 5 and 10 µg/ i.p.	MWM	↓EL, ↑traveled the distance to find the platform, ↓FRAP value, ↓TBARS levels, ↓ GSH-Px activity, ↑SOD	[115]
Rat	Aβ (1–42)	Crocin/ 21 days/i.h.; 150, 300, 600 nmol/ i.p.; 30 mg/kg	MWM	↑traveled distance, ↓EL, swimming speed=>NSD, ↑TSTQ, Beclin-1=>NSD, LC3-II/LC3-I ratio=>NSD, ↓Bax, ↑Bcl-2, ↓Bax/Bcl-2 ratio, ↓ Caspase-3	[116]
Rat/ 50, 5	STZ	Saffron/ 4 weeks/ 20, 40, and 80mg/kg/ i.p.	MWM	↑Bodyweight, ↓ Blood Glucose Levels, ↓EL, ↑TSTQ, ↓TNFα, ↓serum levels of total Lipids, ↓triglycerides, ↓total cholesterol, ↓LDL, ↑ HDL, ↓GSP levels, ↓Serum AGEs, ↑GSH, ↑SOD, ↑CAT, ↓iNOS	[117]
Rat/ 20, 4	STZ	Crocin/21 days/ 100 mg/kg/ p.o.	N.M.	↓MDA levels in the striatum, ↑total thiol, ↑GSH-Px activity	[118]
Rat/ 60, 6	ketamine	Crocins/ 3 days/ 30, 50 mg/kg/ i.p.	NORT	↑discrimination index D, ↓ataxia, total object exploration times=>NSD	[119]
Rat/ 30, 5	ketamine	Crocetin/ 7 days/ 2,4, 8 mg/kg/ i.p.	MWM	↓EL, ↑TSTQ, swimming speed=>NSD, the morphology of neurons in cortex and hippocampus CA1, CA2, CA3 regions=>protected	[120]
Rat/ 48, 8 groups	STZ	Crocin/ 27 days/ 7.5, 15 and 30 mg/kg/ i.p.	EPM	↑Transfer latency, ↓Blood glucose, ↑serum insulin, ↑ TAC, ↓MDA, a decrease of neurons in the hippocampus=> prevented	[121]
Rat/ 35, 5	Quinolinic Acid	Safranal/ 72.75, 145.5, and 291 mg/kg/i.p.	N.M.	(general antioxidant tests were been assessed in vitro), ↓MDA, ↑antioxidant power (FRAP value) of brain homogenate samples, ↑ total thiol content, ↓ DNA damage	[122]
Mouse/ 30, 3	AlCl3	Saffron/ 6 days/ 60 mg/kg/ i.p.	PAT	Step through latency=>NSD, Initial latency=>NSD, ↓SS- and DS-AChE activity in whole brain and cerebellum, cerebral BuChE=>NSD, ↓SS- and DS-liver BuChE and SS-AChE, ↓ MAO-A and MAO-B activity of the whole brain, ↑MAO-B activity of cerebellum, ↓MDA, ↑ GSH	[123]

Animal/ Sample size (No. of groups)	Model of Study	Targeted compound /Duration/ Dosage/ Route of Administration	Cognition Test	Major Outcomes	Ref.
Mouse/ 24, 3	Morphine	Saffron/ 3 days/ 50, 150, and 450 mg/kg/ i.p.	PAT	↑total time spent in the light compartment, ↓total time spent in the dark compartment	[124]
Rat/ 32, 4	STZ	Crocetin in a combination/ 15 days/ 25 µg/kg/ p.o.	MWM, PAT	↑STL, IL=>NSD, ↓ EL, ↑ TSTQ, ↓TBARS level in rat hippocampus and frontal cortex, ↑GSH, ↑GPx, ↑GST, ↑SOD, ↑CAT	[125]
Rat/ 70, 8	chronic cerebral hypoperfusion	5 days/ saffron (50, 100 and 250mg/kg , crocin (5, 10, 25mg/kg/ i.p.	MWM	↓EL, ↑TSTQ	[126]
mouse	D-galactose and NaNO2	Saffron/ 15 days/ 30 mg/kg / i.p.	AAT, PAT	↑avoidance responses, ↑STL	[127]
Mouse/ 39, 4	APP/PS1	Saffron in combination/ 3 months/ 30, 44 mg/kg/ p.o.	MWM	↓EL, ↑TSTQ, cytoplasmic and nuclear membranes=>NSD, ↑cytoplasmic swelling and vacuolation, ↓Aβ immunoreactivity, deformed mitochondrion detected with cristae residues, rare autophagosomes, and dilated Golgi complex and reticulum	[128]
Mouse/ 20, 4	AlCl3, Balb/c and C57BL/6	Saffron/ 45 days/ 200 mg/kg/ i.p.	N.M.	↑SOD, ↑CAT, ↑GSH-Px, ↑Total antioxidants capacity, ↓ TBARS, ↓Arginase, ↓a-l-fucosidase, ↑R-spondin gene, inositol polyphosphate phosphatase-like gene, Bcl-2=>abscent	[129]
Mouse/ 16, 2	Balb-c, aging	Saffron/ 6 days/ 60mg/kg/ i.p.	PAT	IL=>NSD, ↑STL, ↓ SS and DS AChE-specific activity, ↓MDA, ↑ GSH, ↓caspase-3	[130]
Rat/ 150, 8	chronic stress	21 days/ Crocin; 15, 30 mg/kg, Saffron; 30 mg/kg/ i.p.	MWM	↓corticosterone levels, ↓ EL, ↑TSTQ, swimming speed=>NSD, ↑FRAP levels, ↓TBARS levels, ↓SOD, ↓GSH, ↓ GSH-Px	[131]
Rat/ 45, 3	STZ	Saffron/ 3 weeks/ 30 mg/kg/ i.p.	PAT	↓IL, ↑STL, patohistolocal effects=>NSD	[132]
Rat/ 90, 6	STZ	Crocin/ 2 days/ 15 and 30 mg/kg/ i.p.	Y-maze, PAT	↓body weight, ↑step-through latency, ↑(%)Alternation behavior, ↑No. of animals falling	[133]
Rat/ 60, 5	STZ	Saffron/ 3 weeks/ 60mg/kg/ i.p.	Y-maze, PAT	↓IL, ↑STL, ↑(%)scores of Alternation behavior, ↑Psychomotor coordination (PMC) index	[134]
Rat/NORT;40,4/PAT; 80, 8	scopolamine	Saffron/ 10, 30, 60 mg/kg/ i.p.	NORT, PAT	Total motor activity=>NSD, Total exploration time=>NSD, ↑exploration time of the novel object, ↑Discrimination index D, ↑Retention latencies to enter the dark chamber	[135]
Rat	acetaldehyde	Saffron/ 62.5, 125, 250 mg/kg/ p.o.	N.M.	inhibition of long-term potentiation (LTP) in the rat dentate gyros=>inhibited	[136]
Mouse	ethanol	Crocin/ 50, 100, 200 mg/kg/ p.o.	PAT	↓No. of errors, ↑STL	[137]

Table 2. Results based on *in vitro* assays.

Targeted Cell Lines	Model	Targeted Compound/ Exposure Time/ Dosage	Major outcomes	Ref.
PC12	Aluminum maltolate	Saffron/ 48 hours/ 50 and 100 µg/ml	↑cell viability, ↓ total apoptotic cells, ↓CAT activity	[138]

Targeted Cell Lines	Model	Targeted Compound/ Exposure Time/ Dosage	Major outcomes	Ref.
PC12	Aβ(25–35), H2O2	saffron extract, essential oil, safranal/ 120 min/ saffron extract, essential oil, safranal (2.5, 5, 10, 20, 40, 80 μM)	↑cell survival rates, ↓fluorescence intensity, ↓ROS production, ↓ amount of apoptosis, ↓Cyt, and cleaved caspase 3, increased phosphor SAPK/JNK to SAPK/JNK=>inverted, reduction in surviving and the ratio of Phospho-p44/42 MAPK (ERK1/2) to p44/42 MAPK (ERK1/2) and increase in Phospho-PI3 kinase p85/p55 to PI3 kinase p85/p55=>protected	[139]
HT22	L-glutamate (L-Glu)	Crocin/ 3 h/ 0.5 and 2 μM	L-Glu-induced mitochondrial apoptosis in HT22 cells against =>protected, MMP dissipation=>restored, hyper-levels of intracellular ROS compared with the L-Glu-damaged HT22 cells=>suppressed, an overload of Ca2+=>inhibited, Bcl-xL, Bax, Bad, caspase-3=>restored to standard levels, attenuation the phosphorylation of Akt and mTOR=>reversed	[81]
N.M.	N.M.	Crocin/ 50 -800 μM	AChE; IC50=0.92±0.23	[140]
SH-SY5Y, PC12	SH-SY5Y-APP, PC12-htau	trans-Crocin 4, trans-Crocetin/ 24, 72 hours/ 0.1, 1, 10, 100, 1000 μM	cell viability=>NSD, Trans-crocin 4; ↓ total PSEN1,↓ (γ-secretases)PSEN1 and PSEN2 complexes, ↑PSEN1-CTF and PSEN2, ↓ (β-secretase)BACE1, sAPPα↓, APP-C83=>NSD, ↓APP-C99 Trans-crocetin; total APP, ↑ cellular APP and APP-C99 trans-crocin 4; total tau levels↓,↓ tau phosphorylation (pThr231 and pSer199/Ser202-tau), active and inactive forms of GSK3β (total GSK3β, pSer9-GSK3β) and ERK1/2 (total ERK2, pERK1, pERK2)=>downregulated, ERK1=>NSD, trans-crocetin; ↓total and phosphorylated tau (pThr231 and pSer199/Ser202), as well as GSK3β, ERK2, pERK1 and pERK2, ↑inactive pSer9-GSK3β	[141]
HeLa cells	APP751	Crocetin/ 8 h/ 10, 20, 40 μM	cell viability=>NSD, ↓Aβ42 and Aβ40	[93]
N.M.	N.M.	saffron, crocin-1, crocin-2, crocetin, and safranal), and crocetin structural analogs/ 0.233, 0.669, 2.33, 6.99, 23.3 μg/mL	Saffron; inhibited αS aggregation and dissociated αS fibril; IC50=0.656 and EC50=4.17 μg/mL, fibrils of αS↓, Crocin-1, crocin-2, crocetin, and norbixin inhibited αS aggregation and dissociated αS fibrils, with IC50 values of 4.00, 0.541, 0.0930 (first), and 0.911 μM, respectively, and EC50 values of 4.95, 3.63, 0.0617 (first), and 0.244 μM/ safranal, hexadecanedioic acid, and trans, trans-muconic acid; did not show >50% inhibition and >50% efficacy	[142]
LS-180, SH-SY5Y, and THP-1	NLRP3 inflammasom	IIIM-141/ 100 μg/mL	IL-1β=>inhibited, ↑efflux of the Rh123 dye, ↓% intracellular Rh123 levels in LS-180 cells, ↑protection of SH-SY5Y cells from amyloid-β toxicity, and protection of SH-SY5Y cells from glutamate toxicity, ↑expression of P-gp and the amyloid-β clearance	[100]

Targeted Cell Lines	Model	Targeted Compound/ Exposure Time/ Dosage	Major outcomes	Ref.
bEnd3	BBB endothelium	Saffron/ 1.1, 2.2 µg/mL	↑tightness of bEnd-3 cells-based BBB model, ↑transport of 125I-Aβ40 from basolateral to apical compartment across the bEnd-3 cells, ↑ P-gp expression, LRP1 expression=>NSD	[109]
AD patients' monocytes	Aβ1-42	trans-crocetin/ 24, 48, 72, and 120 h/ 5, 10, 25, 50, 100, 150 µM	↓cell death, ↑Aβ42 degradation, ↑Aβ42 degradation through CatB	[143]
EA.hy926	H2O2	Saffron in a combination/ 24 h/ 8.25, 16.5, 33 mg/kg	↓ROS-related cellular damage, ↓LDH leakage,↑ SOD, Intracellular ROS Generation=>suppressed, ↓Bax/Bcl-2 ratio, ↓cleaved caspase-3 expression	[144]
NSCs	Aβ1-42	Saffron/ 26 h/ 25 µg/ml	↑nuclear intensities of caspase-3 activation in Aβ-treated samples,↑ total No. of ROIs indicated expression of c-fos concerning their nuclei	[145]
SH-SY5Y	Aβ	Crocin/ 24 h 1, 10, 50 µM	↓fluorescence intensity, ↑cell viability, intracellular ROS level=>inhibited, formation of Aβ fibril=>inhibited	[146]
N.M.	N.M.	Saffron/ 50, 250 µg/mL	β-secretase=>inhibited	[147]
N.M.	N.M.	Saffron pollens in a combination in alcoholic and nonalcoholic beverage	Nonalcoholic; DPPH=> 91.95 ± 0.23 (mg trolox/100 mL beverages) , FRAP=> 2.51 ± 0.01 (mmol Fe(II)/100 mL beverages) Alcoholic; DPPH=> 66.19 ± 0.17, FRAP=> 1.28 ± 0.02/ ↓AchE	[148]
PC12 cells	1N/4R human tau protein	Crocin /24 h/ 100 µg/mL	tau aggregation=>inhibited	[149]
HT22 cells	H2O2, Aβ1-42	Crocetin/ 24 h/ 3 µM	HT22 cells H2O2-induced cell death=>protected, HT22 cells Aβ1-42-induced cell death=> protected, ↓ROS production induced by Aβ1-42	[150]
N.M.	Aβ42	Crocin/ 15.4 µM	↓fluorescence intensity (degree of amyloid assembly), ↓ANS fluorescence intensity (hydrophobic surface exposure of Aβ42), ↓number of fibrils	[151]
N.M.	Aβ1–40	Crocin /15 µg	↓ThT fluorescence intensity, ↓ANS fluorescence, ↓number, and average fibril length	[152]
N.M.	N.M.	Saffron	↓AChE/ IC50 of crocetin, dimethylcrocetin, safranal=>96.33, 107.1, and 21.09 µM respectively	[153]
SH-SY5Y	H2O2	Saffron; 1, 10, 50 125, 250 µg/mL/ Crocetin; 0.1, 1, 10, 50, 125 µM/ Safranal; 0.1, 1, 10, 50, 125 µM/ 18 h	H2O2-induced toxicity on SH-SY5Y(saffron, safranal, crocetin)=>prevented, rescuing cell viability, ↓ROS production, ↓H2O2-induced caspase-3 activation	[130]
N.M.	Aβ	Crocetin	ThT fluorescence=>NSD, A11-positive conformational epitopes of Aβ oligomers=>preserved	[154]
N.M.	α-Lactalbumin	Crocin, Safranal/ 50, 100 µM	↓fluorescence intensity, ↓ANS fluorescence intensity, ↑cell viability	[155]
BV2 microglial cells	(LPS), Aβ25-35 + IFN-γ	Crocin, crocetin/ 24 h/5, 10, 20 µM	↓NO production, ↑cell viability, ↓TNF-α, ↓ IL-1β,↓ ROS production, ↓NF-κB activation, neuronal damage=>protected	[156]
N.M.	N.M.	saffron	AChE=>NSD	[157]
N.M.	Aβ1-40	saffron, crocin	antioxidant activity=>observed , ↓fluorescence intensity	[158]
PC-12 cells	TNF-α	Crocin/ 24 h/ 1, 10 µM	↓expression of Bcl-2, morphological changes and DNA fragmentation=>blocked	[159]
hippocampal slices	ethanol	Crocin/ 10 µM	inhibition of non-NMDA response=>NSD, inhibition of NMDA response=>significantly blocked	[160]

Targeted Cell Lines	Model	Targeted Compound/ Exposure Time/ Dosage	Major outcomes	Ref.
hippocampal slices	ethanol	Crocin/ 10, 20, 30 μM	↑Spike amplitude, impairment of hippocampal synaptic plasticity=>prevented	[161]

Table 3. Results of clinical trials.

Clinical Trial Type	Patients' Demographic features	Compound/ Route of Administration/ Duration Dosage	test	Major outcomes	Ref.
Randomized Double-Blind Placebo-Controlled Trial	Saffron; N=18 Placebo; N=19 Patients <70 years and who had aWMS-R score >70	saffron capsules/ p.o./ 12 weeks 15 mg/twice daily	WMS-R, HADS, MMSE	NSD in scores	[162]
Randomized Double-Blind Clinical Trial	Herbal combination; N=30 Placebo; N=30, diagnosed MCD patients, mean age of patients =66.8 years, mean age of placebo= 70.2 years	Saffron in a combination/ p.o./ 4, 8 weeks (each capsule containing 500 mg of sedge and 30 mg of saffron extract) plus a teaspoon of Astragalus honey (equal to 5 g in each take)/ 2 capsules per day	ACE, GDS	Attention, memory, language, and visuospatial function=> significant changes, fluency variable=>NSD, MMSE, ACE, GDS scores=>significant changes	[163]
Pilot, Double-Blind, Randomized Clinical Trial	30 young elderly subjects (mean age 66±3), MMSE=between 20 and 27	Saffron in a combination/ p.o./ 8 weeks/ Combined nutraceutical	MMSE, PSQ Index, SRDS	MMSE=>NSD, ↓PSQ Index, ↓SRDS	[164]
Single-Blind Randomized, with Parallel Groups, Clinical Trial	patient; N=17 control; N=18 mean age of patients with MDI= 71.47 mean age of control= 69.72	Saffron/ 12 months	GDS, FRSSD, MMSE, NPI, MOCA MRI, EEG, and ERP	MMSE=>NSD, ↓FRSSD, MoCA=>NSD, ↑NPI, GDS=>NSD, MRI, EEG, and ERP=> showed improvement in specific domains	[165]
Phase 2 Clinical Trial, Randomized, Multicenter, Double-Blind, Placebo-Controlled Study	male and female patients, 40 years of age or older, with a diagnosis of probable mild to moderate VaD	Saffron in a combination/ 26 weeks	V-ADAS-cog, ADCS-CGIC), MMSE, CDR, ADCS-ADL, CLOX, EXIT25, NPI, MRI or CT	The results of the study will provide high-quality evidence on the effect of SLT capsule in patients with VaD and have the potential to establish a novel therapeutic approach for VaD	[166]
Double-Blind Randomized Clinical Trial	Male and female outpatients older than 60 years, MMSE score of 8–14 at baseline Saffron; N=34 Memantine; N=34	Saffron/ p.o./ 12 months/ 30 mg/day	SCIRS, FAST, MMSE	Scores=>NSD between memantine and saffron groups	[167]
multicenter, randomized, double-blind controlled trial	MMSE; 15–26 inclusive 54 Persian-speaking adults	Saffron / p.o./ 15 mg for 4 weeks/ 22 weeks	ADAS-cog, CDR-SB	Scores=>NSD	[168]

	55 years of age or older				
Randomized and Placebo-Controlled Trial	56 patients older than 55 years , MMSE) score of 15–26	Saffron capsules/ p.o./ 16-week/ 15 mg twice per day	ADAS-cog, CDR-SB	ADAS-cog, CDR-SB=>significant changes	[169]

4. Pharmacological and Pharmaceutical Aspects of Saffron

From the collected results, it is concluded that saffron and its active constituents may be a great therapeutic agent combat cognitive dysfunctions. Toxic and lethal effects of saffron can be revealed in 5g and 20 g of the daily dose, respectively. Therapeutic activities of saffron have been observed in (30-50mg/day) clinically; thus, saffron considered as a medicinal plant that possesses a wide therapeutic index (145)[170]. Clinical safety evaluation of crocin through a placebo-controlled, double-blind, randomized study has shown a safe and well-tolerated manifestation in healthy volunteers administered 20 mg/day of crocin in one month [171].

Through studying the pharmacokinetic properties of saffron, it is indicated that oral administration of crocetin is a water-soluble carotenoid of saffron stigmas crocetin is absorbed and detected in plasma faster than the other carotenoids, through its excretion take place widely through the intestinal tract, plasma concentrations of crocetin are not accumulated following repeated doses crocin, hydrolysis of crocin is taken place mostly in the intestinal tract. Crocetin can penetrate the blood barrier brain (BBB) and reach to CNS; it has a high affinity to the NMDA receptor; thus, saffron applied in the treatment of CNS disorders [172,173]. The free form of crocetin may be bound to albumin in blood plasma, this weak bind cand break easily, and crocetin may be able to penetrate the tissues. To enhance the stability of crocin, chitosan, and alginate biopolymer carriers can be used for crocin delivery and encapsulation of bioactive agents [29,174].

5. Conclusions

Various health complications can be treated by the application of medicinal plants, phytochemicals, and nutraceuticals. Discovery and identification of these components with possession of numerous clinical indications can ameliorate our lives quality. Saffron and its active phytochemicals comprising crocin, crocetin, and safranal have the capacity to elucidate anti-AD and memory enhancer properties. Mostly common contributed mechanisms to combat AD are underlying oxidative stress, inflammatory process, apoptosis, beta-amyloid aggregation, and tauopathy. Saffron can be used in the prevention and treatment of clinical complications with etiologies the same as AD. Moreover, regarding the capability of saffron to penetrate BBB it can be considered in therapeutic approaches of other neurological disorders. Study particular cell signaling pathways contributed in AD is recommended following saffron administration. From all of the mentioned extracted data of original researches, memory-enhancing properties of saffron are concluded. However, some modifications in carriers, bioavailability, and route of administration will be required.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Ibrahim, M.M.; Gabr, M.T. Multitarget therapeutic strategies for Alzheimer's disease. *Neural Regen Res* **2019**, *14*, 437-440, https://doi.org/10.4103/1673-5374.245463.
2. Shah, H.; Albanese, E.; Duggan, C.; Rudan, I.; Langa, K.M.; Carrillo, M.C.; Chan, K.Y.; Joanette, Y.; Prince, M.; Rossor, M.; Saxena, S.; Snyder, H.M.; Sperling, R.; Varghese, M.; Wang, H.; Wortmann, M.; Dua, T. Research priorities to reduce the global burden of dementia by 2025. *Lancet Neurol* **2016**, *15*, 1285-1294, https://doi.org/10.1016/s1474-4422(16)30235-6.
3. Noroozian, M. Alzheimer's Disease. *Neurol Clin* **2016**, *34*, 69-131, https://doi.org/10.1016/j.ncl.2015.08.005.
4. International, A.S.D. World Alzheimer Report 2019: Attitudes to dementia. *London: Alzheimer's Disease International* **2019**.
5. Meraz-Ríos, M.A.; Toral-Rios, D.; Franco-Bocanegra, D.; Villeda-Hernández, J.; Campos-Peña, V. Inflammatory process in Alzheimer's Disease. *Front Integr Neurosci* **2013**, *7*, 59-59, https://doi.org/10.3389/fnint.2013.00059.
6. Roher, A.; Kokjohn, T.; Clarke, S.; Sierks, M.; Maarouf, C.; Serrano, G.; Sabbagh, M.; Beach, T. APP/Aβ structural diversity and Alzheimer's disease pathogenesis. *Neurochem Int* **2017**, *110*, https://doi.org/10.1016/j.neuint.2017.08.007.
7. Du, S.; Readel, E.R.; Wey, M.; Armstrong, D.W. Complete identification of all 20 relevant epimeric peptides in β-amyloid: a new HPLC-MS based analytical strategy for Alzheimer's research. *Chem Commun* **2020**, *56*, 1537-1540, https://doi.org/10.1039/C9CC09080K.
8. Rajendran, L.; Honsho, M.; Zahn, T.R.; Keller, P.; Geiger, K.D.; Verkade, P.; Simons, K. Alzheimer's disease β-amyloid peptides are released in association with exosomes. *Proc Natl Acad Sci* **2006**, *103*, 11172-11177, https://doi.org/10.1073/pnas.0603838103.
9. Nandakumar, A.; Xing, Y.; Aranha, R.R.; Faridi, A.; Kakinen, A.; Javed, I.; Koppel, K.; Pilkington, E.H.; Purcell, A.W.; Davis, T.P.; Faridi, P.; Ding, F.; Ke, P.C. Human Plasma Protein Corona of Aβ Amyloid and Its Impact on IAPP Cross-Seeding. *Biomacromolecules* **2020**, *21*, 988-998, https://doi.org/10.1021/acs.biomac.9b01650.
10. Karch, C.M.; Cruchaga, C.; Goate, A.M. Alzheimer's disease genetics: from the bench to the clinic. *Neuron* **2014**, *83*, 11-26, https://doi.org/10.1016/j.neuron.2014.05.041.
11. Oliver, D.; Reddy, P.H. Small molecules as therapeutic drugs for Alzheimer's disease. *Mol Cell Neurosci* **2019**, *96*, https://doi.org/10.1016/j.mcn.2019.03.001.
12. Näslund, J.; Haroutunian, V.; Mohs, R.; Davis, K.L.; Davies, P.; Greengard, P.; Buxbaum, J.D. Correlation Between Elevated Levels of Amyloid β-Peptide in the Brain and Cognitive Decline. *JAMA* **2000**, *283*, 1571-1577, https://doi.org/10.1001/jama.283.12.1571.
13. Aprahamian, I.; Stella, F.; Forlenza, O. New treatment strategies for Alzheimer's disease: Is there a hope? *Indian J Med Res* **2013**, *138*, 449-460.
14. Kozauer, N.; Katz, R. Regulatory innovation and drug development for early-stage Alzheimer's disease. *N Engl J Med* **2013**, *368*, 1169-1171, https://doi.org/10.1056/nejmp1302513.
15. Vaisi-Raygani, A.; Rahimi, Z.; Zahraie, M.; Noroozian, M.; Pourmotabbed, A. Association between enzymatic and nonenzymatic antioxidant defense with Alzheimer disease. *Acta Med Iran* **2008**, *46*, 11-16.
16. Manayi, A.; Saeidnia, S.; Gohari, A.R.; Abdollahi, M. Methods for the discovery of new anti-aging products – targeted approaches. *Expert Opin Drug Discov* **2014**, *9*, 383-405, https://doi.org/10.1517/17460441.2014.885014.
17. Small, G.W.; Ercoli, L.M.; Silverman, D.H.; Huang, S.C.; Komo, S.; Bookheimer, S.Y.; Lavretsky, H.; Miller, K.; Siddarth, P.; Rasgon, N.L. Cerebral metabolic and cognitive decline in persons at genetic risk for Alzheimer's disease. *Proc Natl Acad Sci* **2000**, *97*, 6037-6042, https://doi.org/10.1073/pnas.090106797.
18. Farkhondeh, T.; Azimi-Nezhad, M.; Samini, F.; Pourbagher-Shahri, A.M.; Samarghandian, S. Neuroprotective effect of alpinia galanga against middle cerebral artery occlusion-induced ischemia in rat. *Biointerface Res Appl Chem* **2020**, *10*, 6273-6281.

19. Rosini, M.; Simoni, E.; Minarini, A.; Melchiorre, C. Multi-target Design Strategies in the Context of Alzheimer's Disease: Acetylcholinesterase Inhibition and NMDA Receptor Antagonism as the Driving Forces. *Neurochem Res* **2014**, *39*, 1914-1923, https://doi.org/10.1007/s11064-014-1250-1.
20. Hampel, H. Current insights into the pathophysiology of Alzheimer's disease: selecting targets for early therapeutic intervention. *Int Psychogeriatr* **2012**, *24*, S10-S17, https://doi.org/10.1017/s1041610212000579.
21. Yazdani, E.; Talebi, M.; Zarshenas, M.; Moein, M. Evaluation of possible antioxidant activities of barberry solid formulation, a selected formulation from Traditional Persian Medicine (TPM) via various procedures. *Biointerface Res Appl Chem* **2019**, *9*, 4517-4521, https://doi.org/10.33263/BRIAC96.517521.
22. Talebi, M.; Zarshenas, M.M.; Moein, M.; Yazdani, E. Experimental evidence of the antioxidant effect of a multi-ingredient traditional Persian formulation with hepatoprotective activity. In: Proceedings of 12th International Congress Laboratory and Clinical Sciences, Tehran, Iran; pp. 931-932.
23. Howes, M.J.R.; Perry, E. The Role of Phytochemicals in the Treatment and Prevention of Dementia. *Drugs Aging* **2011**, *28*, 439-468, https://doi.org/10.2165/11591310-000000000-00000.
24. Russo, P.; Frustaci, A.; Bufalo, A.D.; Fini, M.; Cesario, A. Multitarget drugs of plants origin acting on alzheimer's disease. *Curr Med Chem* **2013**, *20*, 1686-1693, https://doi.org/10.2174/0929867311320130008.
25. Alavizadeh, S.H.; Hosseinzadeh, H. Bioactivity assessment and toxicity of crocin: a comprehensive review. *Food Chem Toxicol* **2014**, *64*, 65-80, https://doi.org/10.1016/j.fct.2013.11.016.
26. Akhondzadeh, S. Crocus sativus (saffron), an herb with a history as long as the history of iran with psychotropic effects. *J Med Plants* **2016**, *15*, 1-6.
27. Farkhondeh, T.; Azimi-Nezhad, M.; Samini, F.; Pourbagher-Shahri, A.M.; Samarghandian, S. Neuroprotective effect of alpinia galanga against middle cerebral artery occlusion-induced ischemia in rat. *Biointerface Res Appl Chem* **2020**, *10*, 6273-6281.
28. Rezaee, R.; Hosseinzadeh, H. Safranal: from an aromatic natural product to a rewarding pharmacological agent. *Iran J Basic Med Sci* **2013**, *16*, 12-26.
29. Rahaiee, S.; Hashemi, M.; Shojaosadati, S.A.; Moini, S.; Razavi, S.H. Nanoparticles based on crocin loaded chitosan-alginate biopolymers: Antioxidant activities, bioavailability and anticancer properties. *Int J Biol Macromol* **2017**, *99*, 401-408, https://doi.org/10.1016/j.ijbiomac.2017.02.095.
30. Gohari, A.; Saeidnia, S.; kurepaz mahmoodabadi, M. An overview on saffron, phytochemicals, and medicinal properties. *Pharmacogn Rev* **2013**, *7*, 61-66, https://doi.org/10.4103/0973-7847.112850.
31. Talebi, M.; Patil, R.; Sidisky, L.; Berthod, A.; Armstrong, D. Branched-chain dicationic ionic liquids for fatty acid methyl ester assessment by gas chromatography. *Anal Bioanal Chem* **2017**, *410*, 1-11, https://doi.org/10.1007/s00216-017-0722-y.
32. Samarghandian, S.; Borji, A. Anticarcinogenic effect of saffron (Crocus sativus L.) and its ingredients. *Pharmacognosy Res* **2014**, *6*, 99-107, https://doi.org/10.4103/0974-8490.128963.
33. Bhandari, P.R. Crocus sativus L.(saffron) for cancer chemoprevention: a mini review. *J Tradit Complement Med* **2015**, *5*, 81-87, https://doi.org/10.1016/j.jtcme.2014.10.009.
34. Soeda, S.; Aritake, K.; Urade, Y.; Sato, H.; Shoyama, Y. Neuroprotective Activities of Saffron and Crocin. *Adv Neurobiol* **2016**, *12*, 275-292, https://doi.org/10.1007/978-3-319-28383-8_14.
35. Yazdi, H.; Samarghandian, S.; Farkhondeh, T. Effects of Crocins in the Management of Neurodegenerative Pathologies: A Review. *Neurophysiology* **2018**, *50*, https://doi.org/10.1007/s11062-018-9752-0.
36. Pitsikas, N. The effect of Crocus sativus L. and its constituents on memory: basic studies and clinical applications. *Evid Based Complement Alternat Med* **2015**, *2015*, https://doi.org/10.1155/2015/926284.
37. Farkhondeh, T.; Samarghandian, S.; Yazdi, H.; Samini, F. The protective effects of crocin in the management of neurodegenerative diseases: a review. *Am J Neurodegener Dis* **2018**, *7*, 1-10.
38. Hosseinzadeh, H. Saffron: A Herbal Medicine of Third Millennium. *Jundishapur J Nat Pharm Prod* **2014**, *9*, 1-2.
39. Baba, S.A.; Malik, A.H.; Wani, Z.A.; Mohiuddin, T.; Shah, Z.; Abbas, N.; Ashraf, N. Phytochemical analysis and antioxidant activity of different tissue types of Crocus sativus and oxidative stress alleviating potential of saffron extract in plants, bacteria, and yeast. *S Afr J Bot.* **2015**, *99*, 80-87, https://doi.org/10.1016/j.sajb.2015.03.194.
40. Farkhondeh, T.; Samarghandian, S. The effect of saffron (Crocus sativus L.) and its ingredients on the management of diabetes mellitus and dislipidemia. *Afr J Pharm Pharmacol* **2014**, *8*, 541-549, https://doi.org/10.5897/AJPPX2013.0006.
41. Rahmani, A.; Khan, A.; Aldebasi, Y. Saffron (Crocus sativus) and its Active Ingredients: Role in the Prevention and Treatment of Disease. *Pharmacognosy J* **2017**, *9*, 873-879, https://doi.org/10.5530/pj.2017.6.137.
42. Kamalipour, M.; Akhondzadeh, S. Cardiovascular effects of saffron: an evidence-based review. *J Tehran Heart Cent* **2011**, *6*, 59-61.
43. Khorasany, A.R.; Hosseinzadeh, H. Therapeutic effects of saffron (Crocus sativus L.) in digestive disorders: a review. *Iran J Basic Med Sci* **2016**, *19*, 455-469.
44. Hosseinzadeh, H.; Younesi, H.M. Antinociceptive and anti-inflammatory effects of Crocus sativus L. stigma and petal extracts in mice. *BMC Pharmacol* **2002**, *2*, https://dx.doi.org/10.1186%2F1471-2210-2-7.

45. Khazdair, M.R.; Boskabady, M.H.; Hosseini, M.; Rezaee, R.; Tsatsakis, A.M. The effects of Crocus sativus (saffron) and its constituents on nervous system: A review. *Avicenna J Phytomed* **2015**, *5*, 376-391.
46. Kyriakoudi, A.; Ordoudi, S.; Roldán-Medina, M.; Tsimidou, M. Saffron, a functional spice. *Austin J Nutri Food Sci* **2015**, *3*.
47. Shahi, T.; Assadpour, E.; Jafari, S.M. Main chemical compounds and pharmacological activities of stigmas and tepals of 'red gold'; saffron. *Trends Food Sci Technol* **2016**, *58*, 69-78, https://doi.org/10.1016/j.tifs.2016.10.010.
48. Bhargava, V. Medicinal uses and pharmacological properties of Crocus sativus Linn (Saffron). *Int. J. Pharm. Pharm. Sci* **2011**, *3*, 22-26.
49. Liberati, A.; Altman, D.G.; Tetzlaff, J.; Mulrow, C.; Gøtzsche, P.C.; Ioannidis, J.P.A.; Clarke, M.; Devereaux, P.J.; Kleijnen, J.; Moher, D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ* **2009**, *339*, b2700, https://doi.org/10.1136/bmj.b2700.
50. Hölter, S.; Garrett, L.; Einicke, J.; Sperling, B.; Dirscherl, P.; Zimprich, A.; Fuchs, H.; Gailus-Durner, V.; Angelis, M.; Wurst, W. Assessing Cognition in Mice. *Curr Protoc Mouse Biol* **2015**, *5*, 331-358, https://doi.org/10.1002/9780470942390.mo150068.
51. Farkhondeh, T.; Samarghandian, S; Sedaghat, M. Agents-induced nephrotoxicity and catcheins. *Biointerface Res Appl Chem* **2020**, *10*, 5028-5031.
52. Yu, T.; Guo, M.; Garza, J.; Rendon, S.; Sun, X.L.; Zhang, W.; Lu, X.Y. Cognitive and neural correlates of depression-like behaviour in socially defeated mice: an animal model of depression with cognitive dysfunction. *Int J Neuropsychopharmacol* **2011**, *14*, 303-317, https://doi.org/10.1017/S1461145710000945.
53. Albani, S.H.; Andrawis, M.M.; Abella, R.J.H.; Fulghum, J.T.; Vafamand, N.; Dumas, T.C. Behavior in the elevated plus maze is differentially affected by testing conditions in rats under and over three weeks of age. *Front Behav Neurosci* **2015**, *9*, 31-31, https://doi.org/10.3389/fnbeh.2015.00031.
54. Pocernich, C.B.; Butterfield, D.A. Elevation of glutathione as a therapeutic strategy in Alzheimer disease. *Biochim Biophys Acta Mol Basis Dis* **2012**, *1822*, 625-630, https://doi.org/10.1016/j.bbadis.2011.10.003.
55. Young, A.; Oldford, C.; Mailloux, R.J. Lactate dehydrogenase supports lactate oxidation in mitochondria isolated from different mouse tissues. *Redox Biol* **2020**, *28*, 101339-101339, https://doi.org/10.1016/j.redox.2019.101339.
56. Greilberger, J.; Fuchs, D.; Leblhuber, F.; Greilberger, M.; Wintersteiger, R.; Tafeit, E. Carbonyl Proteins as a Clinical Marker in Alzheimer's Disease and its Relation to Tryptophan Degradation and Immune Activation. *Clin Lab* **2010**, *56*, 441-448.
57. Kudo, W.; Lee, H.P.; Smith, M.A.; Zhu, X.; Matsuyama, S.; Lee, H.g. Inhibition of Bax protects neuronal cells from oligomeric Aβ neurotoxicity. *Cell Death Dis* **2012**, *3*, e309-e309, https://doi.org/10.1038/cddis.2012.43.
58. Walker, D.; Lue, L.F. Anti-inflammatory and immune therapy for Alzheimer's disease: current status and future directions. *Curr Neuropharmacol* **2007**, *5*, 232-243, https://doi.org/157015907782793667.
59. Munoz, L.; Ammit, A.J. Targeting p38 MAPK pathway for the treatment of Alzheimer's disease. *Neuropharmacology* **2010**, *58*, 561-568, https://doi.org/10.1016/j.neuropharm.2009.11.010.
60. Song, J.; Kim, O.Y. Perspectives in Lipocalin-2: Emerging Biomarker for Medical Diagnosis and Prognosis for Alzheimer's Disease. *Clin Nutr Res* **2018**, *7*, 1-10, https://doi.org/10.7762/cnr.2018.7.1.1.
61. Casas, C. GRP78 at the Centre of the Stage in Cancer and Neuroprotection. *Front Neurosci* **2017**, *11*, https://doi.org/10.3389/fnins.2017.00177.
62. Jones, S.V.; Kounatidis, I. Nuclear Factor-Kappa B and Alzheimer Disease, Unifying Genetic and Environmental Risk Factors from Cell to Humans. *Front Immunol* **2017**, *8*, 1805-1805, https://doi.org/10.3389/fimmu.2017.01805.
63. Cunha, C.; Brambilla, R.; Thomas, K.L. A simple role for BDNF in learning and memory? *Front Mol Neurosci* **2010**, *3*, 1-1, https://doi.org/10.3389/neuro.02.001.2010.
64. Ortega-Martínez, S. A new perspective on the role of the CREB family of transcription factors in memory consolidation via adult hippocampal neurogenesis. *Front Mol Neurosci* **2015**, *8*, https://doi.org/10.3389/fnmol.2015.00046.
65. Maia, M.A.; Sousa, E. BACE-1 and γ-Secretase as Therapeutic Targets for Alzheimer's Disease. *Pharmaceuticals (Basel)* **2019**, *12*, 41, https://doi.org/10.3390/ph12010041.
66. Reichenbach, N.; Delekate, A.; Plescher, M.; Schmitt, F.; Krauss, S.; Blank, N.; Halle, A.; Petzold, G.C. Inhibition of Stat3-mediated astrogliosis ameliorates pathology in an Alzheimer's disease model. *EMBO Mol Med* **2019**, *11*, https://doi.org/10.15252/emmm.201809665.
67. Ceyzériat, K.; Ben Haim, L.; Denizot, A.; Pommier, D.; Matos, M.; Guillemaud, O.; Palomares, M.-A.; Abjean, L.; Petit, F.; Gipchtein, P.; Gaillard, M.-C.; Guillermier, M.; Bernier, S.; Gaudin, M.; Aurégan, G.; Joséphine, C.; Déchamps, N.; Veran, J.; Langlais, V.; Cambon, K.; Bemelmans, A.P.; Baijer, J.; Bonvento, G.; Dhenain, M.; Deleuze, J.-F.; Oliet, S.H.R.; Brouillet, E.; Hantraye, P.; Carrillo-de Sauvage, M.-A.; Olaso, R.; Panatier, A.; Escartin, C. Modulation of astrocyte reactivity improves functional deficits in mouse models of Alzheimer's disease. *Acta Neuropathol Commun* **2018**, *6*, 104, https://doi.org/10.1186/s40478-018-0606-1.

68. Tu, S.; Okamoto, S.I.; Lipton, S.A.; Xu, H. Oligomeric Aβ-induced synaptic dysfunction in Alzheimer's disease. *Mol Neurodegener* **2014**, *9*, https://doi.org/10.1186/1750-1326-9-48.
69. Rohn, T.T.; Head, E. Caspases as therapeutic targets in Alzheimer's disease: is it time to "cut" to the chase? *Int J Clin Exp Pathol* **2009**, *2*, 108-118.
70. Verite, J.; Janet, T.; Julian, A.; Chassaing, D.; Page, G.; Paccalin, M. Peripheral blood mononuclear cells of Alzheimer's disease patients control CCL4 and CXCL10 levels in a human blood brain barrier model. *Curr Alzheimer Res* **2017**, *14*, 1215-1228, https://doi.org/10.2174/1567205014666170417110337.
71. Copanaki, E.; Schürmann, T.; Eckert, A.; Leuner, K.; Müller, W.E.; Prehn, J.H.M.; Kögel, D. The amyloid precursor protein potentiates CHOP induction and cell death in response to ER Ca2+ depletion. *Biochim Biophys Acta Mol Cell Res* **2007**, *1773*, 157-165, https://doi.org/10.1016/j.bbamcr.2006.10.002.
72. Stuart, K.E.; King, A.E.; Fernandez-Martos, C.M.; Summers, M.J.; Vickers, J.C. Environmental novelty exacerbates stress hormones and Aβ pathology in an Alzheimer's model. *Sci Rep* **2017**, *7*, 2764-2764, https://doi.org/10.1038/s41598-017-03016-0.
73. Wang, X.X.; Tan, M.S.; Yu, J.T.; Tan, L. Matrix metalloproteinases and their multiple roles in Alzheimer's disease. *Biomed Res Int* **2014**, *2014*, 908636-908636, https://doi.org/10.1155/2014/908636.
74. Zamora-Martinez, E.R.; Edwards, S. Neuronal extracellular signal-regulated kinase (ERK) activity as marker and mediator of alcohol and opioid dependence. *Front Integr Neurosci* **2014**, *8*, 24-24, https://doi.org/10.3389/fnint.2014.00024.
75. Ostrowski, P.P.; Barszczyk, A.; Forstenpointner, J.; Zheng, W.; Feng, Z.P. Meta-Analysis of Serum Insulin-Like Growth Factor 1 in Alzheimer's Disease. *PLoS One* **2016**, *11*, e0155733-e0155733, https://doi.org/10.1371/journal.pone.0155733.
76. Chang, J.R.; Ghafouri, M.; Mukerjee, R.; Bagashev, A.; Chabrashvili, T.; Sawaya, B.E. Role of p53 in neurodegenerative diseases. *Neurodegener Dis* **2012**, *9*, 68-80, https://doi.org/10.1159/000329999.
77. Mohammadzadeh, L.; Abnous, K.; Razavi, B.M.; Hosseinzadeh, H. Crocin-protected malathion-induced spatial memory deficits by inhibiting TAU protein hyperphosphorylation and antiapoptotic effects. *Nutr Neurosci* **2020**, *23*, 221-236, https://doi.org/10.1080/1028415x.2018.1492772.
78. Oddo, S. The role of mTOR signaling in Alzheimer disease. *Front Biosci (Schol Ed)* **2012**, *4*, 941-952, https://doi.org/10.2741/s310.
79. Karkoula, E.; Dagla, I.V.; Baira, E.; Kokras, N.; Dalla, C.; Skaltsounis, A.L.; Gikas, E.; Tsarbopoulos, A. A novel UHPLC-HRMS-based metabolomics strategy enables the discovery of potential neuroactive metabolites in mice plasma, following i.p. administration of the main Crocus sativus L. bioactive component. *J Pharm Biomed Anal* **2020**, *177*, https://doi.org/10.1016/j.jpba.2019.112878.
80. Zhang, Y.; Liu, J.; Yao, M.; Song, W.; Zheng, Y.; Xu, L.; Sun, M.; Yang, B.; Bensoussan, A.; Chang, D.; Li, H. Sailuotong Capsule Prevents the Cerebral Ischaemia-Induced Neuroinflammation and Impairment of Recognition Memory through Inhibition of LCN2 Expression. *Oxid Med Cell Longev* **2019**, *2019*, https://doi.org/10.1155/2019/8416105.
81. Wang, C.; Cai, X.; Hu, W.; Li, Z.; Kong, F.; Chen, X.; Wang, D. Investigation of the neuroprotective effects of crocin via antioxidant activities in HT22 cells and in mice with Alzheimer's disease. *Int J Mol Med* **2019**, *43*, 956-966, https://doi.org/10.3892/ijmm.2018.4032.
82. Shahidani, S.; Rajaei, Z.; Alaei, H. Pretreatment with crocin along with treadmill exercise ameliorates motor and memory deficits in hemiparkinsonian rats by anti-inflammatory and antioxidant mechanisms. *Metab Brain Dis* **2019**, *34*, 459-468, https://doi.org/10.1007/s11011-018-0379-z.
83. Sadoughi, D. The effect of crocin on apoptotic, inflammatory, BDNF, Pt, and Aβ40 indicators and neuronal density of CA1, CA2, and CA3 regions of hippocampus in the model of Alzheimer suffering rats induced with trimethyltin chloride. *Comp Clin Path* **2019**, *28*, 1403-1413, https://doi.org/10.1007/s00580-019-02981-4.
84. Negarandeh, Z.; Salamat, K.M.; Ali Hosseini, S.; Etemad, Z. The effect of endurance training with crocin consumption on IGF-1 and glycogen expression in rat hippocampus tissue of trimethyltin-treated model of Alzheimer's disease. *Asian J Sports Med* **2019**, *10*, https://doi.org/10.5812/asjsm.92246.
85. Mozaffari, S.; Ramezany Yasuj, S.; Motaghinejad, M.; Motevalian, M.; Kheiri, R. Crocin Acting as a Neuroprotective Agent against Methamphetamine-induced Neurodegeneration via CREB-BDNF Signaling Pathway. *Iran J Pharm Res: IJPR* **2019**, *18*, 745-758, https://doi.org/10.22037/ijpr.2019.2393.
86. Lin, L.; Liu, G.L.; Yang, L.N. Crocin Improves Cognitive Behavior in Rats with Alzheimer's Disease by Regulating Endoplasmic Reticulum Stress and Apoptosis. *Biomed Res Int* **2019**, *2019*, https://doi.org/10.1155/2019/9454913.
87. Ebrahimzadeh, A.; Moghadam, S.Y.; Rahimi, H.; Motaghinejad, M.; Motevalian, M.; Safari, S.; Mesrabadi, M.A. Crocin acts as a neuroprotective mediator against methylphenidate-induced neurobehavioral and neurochemical sequelae: Possible role of the CREB-BDNF signaling pathway. *Acta Neurobiol Exp* **2019**, *79*, 352-366.
88. Baluchnejadmojarad, T.; Mohamadi-Zarch, S.M.; Roghani, M. Safranal, an active ingredient of saffron, attenuates cognitive deficits in amyloid β-induced rat model of Alzheimer's disease: underlying mechanisms. *Metab Brain Dis* **2019**, *34*, 1747-1759, https://doi.org/10.1007/s11011-019-00481-6.

89. Azarian, F.; Farsi, S.; Hosseini, S.A.; Azarbayjani, M.A. The effect of endurance training and crocin consumption on anxiety-like behaviors and aerobic power in rats with alzheimer's. *Iran J Psychiatry Behav Sci* **2019**, *13*, https://doi.org/10.5812/ijpbs.89011.
90. Asalgoo, S.; Jahromi, G.P.; Hatef, B.; Sahraei, H. The effect of saffron aqueous extract and crocin on PTSD rat models: The focus on learning and spatial memory. *Journal of Zanjan University of Medical Sciences and Health Services* **2019**, *26*, 34-42.
91. Adabizadeh, M.; Mehri, S.; Rajabpour, M.; Abnous, K.; Rashedinia, M.; Hosseinzadeh, H. The effects of crocin on spatial memory impairment induced by hyoscine: Role of NMDA, AMPA, ERK and CaMKII proteins in rat hippocampus. *Iran J Basic Med Sci* **2019**, *22*, 601-609, https://doi.org/10.22038/ijbms.2019.30138.7266.
92. Zhang, X.Y.; Zhang, X.J.; Xv, J.; Jia, W.; Pu, X.Y.; Wang, H.Y.; Liang, H.; Zhuoma, L.; Lu, D.X. Crocin attenuates acute hypobaric hypoxia-induced cognitive deficits of rats. *Eur J Pharmacol* **2018**, *818*, 300-305, https://doi.org/10.1016/j.ejphar.2017.10.042.
93. Zhang, J.; Wang, Y.; Dong, X.; Liu, J. Crocetin attenuates inflammation and amyloid-β accumulation in APPsw transgenic mice. *Immun Ageing* **2018**, *15*, https://doi.org/10.1186/s12979-018-0132-9.
94. Rezai, M.; Mahmoodi, M.; Kaeidi, A.; Karimabad, M.N.; Khoshdel, A.; Hajizadeh, M.R. Effect of crocin carotenoid on BDNF and CREB gene expression in brain ventral tegmental area of morphine treated rats. *Asian Pac J Trop Biomed* **2018**, *8*, 387-393, https://doi.org/10.4103/2221-1691.239426.
95. Pitsikas, N.; Tarantilis, P.A. Effects of the active constituents of Crocus sativus L. crocins and their combination with memantine on recognition memory in rats. *Behav Pharmacol* **2018**, *29*, 400-412, https://doi.org/10.1097/FBP.0000000000000380.
96. Khani, F.; Radahmadi, M.; Alaei, H.; Jafari, E. Effects of crocin on cognitive and spatial memories in rats under chronic isolation stress. *Physiol Pharmacol (Iran)* **2018**, *22*, 254-268.
97. Hadipour, M.; Kaka, G.; Bahrami, F.; Meftahi, G.H.; Jahromi, G.P.; Mohammadi, A.; Sahraei, H. Crocin improved amyloid beta induced long-term potentiation and memory deficits in the hippocampal CA1 neurons in freely moving rats. *Synapse* **2018**, *72*, https://doi.org/10.1002/syn.22026.
98. Delkhosh-Kasmaie, F.; Farshid, A.A.; Tamaddonfard, E.; Imani, M. The effects of safranal, a constitute of saffron, and metformin on spatial learning and memory impairments in type-1 diabetic rats: behavioral and hippocampal histopathological and biochemical evaluations. *Biomed Pharmacother* **2018**, *107*, 203-211, https://doi.org/10.1016/j.biopha.2018.07.165.
99. Dastgerdi, H.H.; Radahmadi, M.; Reisi, P.; Dastgerdi, A.H. Effect of Crocin, Exercise, and Crocin-accompanied Exercise on Learning and Memory in Rats under Chronic Unpredictable Stress. *Adv Biomed Res* **2018**, *7*, 137, https://doi.org/10.4103/abr.abr_153_18.
100. Bharate, S.S.; Kumar, V.; Singh, G.; Singh, A.; Gupta, M.; Singh, D.; Kumar, A.; Vishwakarma, R.A.; Bharate, S.B. Preclinical Development of Crocus sativus-Based Botanical Lead IIIM-141 for Alzheimer's Disease: Chemical Standardization, Efficacy, Formulation Development, Pharmacokinetics, and Safety Pharmacology. *Acs Omega* **2018**, *3*, 9572-9585, https://doi.org/10.1021/acsomega.8b00841.
101. Baghishani, F.; Mohammadipour, A.; Hosseinzadeh, H.; Hosseini, M.; Ebrahimzadeh-bideskan, A. The effects of tramadol administration on hippocampal cell apoptosis, learning and memory in adult rats and neuroprotective effects of crocin. *Metab Brain Dis* **2018**, *33*, 907-916, https://doi.org/10.1007/s11011-018-0194-6.
102. Pitsikas, N.; Tarantilis, P.A. Crocins, the active constituents of Crocus sativus L., counteracted apomorphine-induced performance deficits in the novel object recognition task, but not novel object location task, in rats. *Neurosci Lett* **2017**, *644*, 37-42, https://doi.org/10.1016/j.neulet.2017.02.042.
103. Mazumder, A.G.; Sharma, P.; Patial, V.; Singh, D. Crocin Attenuates Kindling Development and Associated Cognitive Impairments in Mice via Inhibiting Reactive Oxygen Species-Mediated NF-B Activation. *Basic Clin Pharmacol Toxicol* **2017**, *120*, 426-433, https://doi.org/10.1111/bcpt.12694.
104. Linardaki, Z.I.; Lamari, F.N.; Margarity, M. Saffron (Crocus sativus L.) Tea Intake Prevents Learning/Memory Defects and Neurobiochemical Alterations Induced by Aflatoxin B1 Exposure in Adult Mice. *Neurochem Res* **2017**, *42*, 2743-2754, https://doi.org/10.1007/s11064-017-2283-z.
105. Heidari, S.; Mehri, S.; Hosseinzadeh, H. Memory enhancement and protective effects of crocin against D-galactose aging model in the hippocampus of wistar rats. *Iran J Basic Med Sci* **2017**, *20*, 1250-1259, https://doi.org/10.22038/IJBMS.2017.9541.
106. Dastgerdi, A.H.; Radahmadi, M.; Pourshanazari, A.A.; Dastgerdi, H.H. Effects of Crocin on Learning and Memory in Rats Under Chronic Restraint Stress with Special Focus on the Hippocampal and Frontal Cortex Corticosterone Levels. *Adv Biomed Res* **2017**, *6*, 157, https://doi.org/10.4103/abr.abr_107_17.
107. Cioancă, O.; Pagonakis, A.; Trifan, A.; Hrițcu, L.; Ioniță, R.; Burlec, A.F.; Postu, P.; Cornelia, M.; Hăncianu, M. Pharmacognostic and pharmacologic screening of crocus sativus of Greek origin. *Farmacia* **2017**, *65*, 401-406.
108. Behravanfar, N.; Abnous, K.; Razavi, B.M.; Hosseinzadeh, H. Effects of crocin on spatial memory impairment induced by hyoscine and its effects on bdnf, creb, and p-creb protein and mrna levels in rat hippocampus. *Jundishapur J Nat Pharm Prod* **2017**, *12*, https://doi.org/10.5812/jjnpp.64315.

109. Batarseh, Y.S.; Bharate, S.S.; Kumar, V.; Kumar, A.; Vishwakarma, R.A.; Bharate, S.B.; Kaddoumi, A. Crocus sativus Extract Tightens the Blood-Brain Barrier, Reduces Amyloid β Load and Related Toxicity in 5XFAD Mice. *ACS Chem Neurosci* **2017**, *8*, 1756-1766, https://doi.org/10.1021/acschemneuro.7b00101.
110. Ahmadi, M.; Rajaei, Z.; Hadjzadeh, M.A.; Nemati, H.; Hosseini, M. Crocin improves spatial learning and memory deficits in the Morris water maze via attenuating cortical oxidative damage in diabetic rats. *Neurosci Lett* **2017**, *642*, 1-6, https://doi.org/10.1016/j.neulet.2017.01.049.
111. Yousefvand, N.; Doosti, H.; Pourmotabbed, A.; Nedaei, S.E. The therapeutic effect of crocin on ketamine-induced retrograde amnesia in rats. *Journal of Kermanshah University of Medical Sciences* **2016**, *20*, 68-73.
112. Rajaei, Z.; Hosseini, M.; Alaei, H. Effects of Crocin on brain oxidative damage and aversive memory in a 6-OHDA model of Parkinson's disease. *Arq Neuropsiquiatr* **2016**, *74*, 723-729, https://doi.org/10.1590/0004-282X20160131.
113. Samarghandian, S.; Azimi-Nezhad, M.; Samini, F. Preventive effect of safranal against oxidative damage in aged male rat brain. *Exp Anim* **2015**, *64*, 65-71, https://doi.org/10.1538/expanim.14-0027.
114. Rashedinia, M.; Lari, P.; Abnous, K.; Hosseinzadeh, H. Protective effect of crocin on acrolein-induced tau phosphorylation in the rat brain. *Acta Neurobiol Exp* **2015**, *75*, 208-219.
115. Ghaffari, S.; Hatami, H.; Dehghan, G. Saffron ethanolic extract attenuates oxidative stress, spatial learning, and memory impairments induced by local injection of ethidium bromide. *Res Pharm Sci* **2015**, *10*, 222-232.
116. Asadi, F.; Jamshidi, A.H.; Khodagholi, F.; Yans, A.; Azimi, L.; Faizi, M.; Vali, L.; Abdollahi, M.; Ghahremani, M.H.; Sharifzadeh, M. Reversal effects of crocin on amyloid β-induced memory deficit: Modification of autophagy or apoptosis markers. *Pharmacol Biochem Behav* **2015**, *139*, 47-58, https://doi.org/10.1016/j.pbb.2015.10.011.
117. Samarghandian, S.; Azimi-Nezhad, M.; Samini, F. Ameliorative effect of saffron aqueous extract on hyperglycemia, hyperlipidemia, and oxidative stress on diabetic encephalopathy in streptozotocin induced experimental diabetes mellitus. *Biomed Res Int* **2014**, *2014*, https://doi.org/10.1155/2014/920857.
118. Naghizadeh, B.; Mansouri, M.T.; Ghorbanzadeh, B. Protective effects of crocin against streptozotocin-induced oxidative damage in rat striatum. *Acta Med Iran* **2014**, *52*, 101-105.
119. Georgiadou, G.; Grivas, V.; Tarantilis, P.A.; Pitsikas, N. Crocins, the active constituents of Crocus Sativus L., counteracted ketamine-induced behavioural deficits in rats. *Psychopharmacology* **2014**, *231*, 717-726, https://doi.org/10.1007/s00213-013-3293-4.
120. Tashakori-Sabzevar, F.; Hosseinzadeh, H.; Motamedshariaty, V.S.; Movassaghi, A.R.; Mohajeri, S.A. Crocetin attenuates spatial learning dysfunction and hippocampal injury in a model of vascular dementia. *Curr Neurovasc Res* **2013**, *10*, 325-334, https://doi.org/10.2174/15672026113109990032.
121. Tamaddonfard, E.; Farshid, A.A.; Asri-Rezaee, S.; Javadi, S.; Khosravi, V.; Rahman, B.; Mirfakhraee, Z. Crocin improved learning and memory impairments in streptozotocin-induced diabetic rats. *Iran J Basic Med Sci* **2013**, *16*, 91-100.
122. Sadeghnia, H.R.; Kamkar, M.; Assadpour, E.; Boroushaki, M.T.; Ghorbani, A. Protective effect of safranal, a constituent of crocus sativus, on quinolinic acid-induced oxidative damage in rat hippocampus. *Iran J Basic Med Sci* **2013**, *16*, 73-82.
123. Linardaki, Z.I.; Orkoula, M.G.; Kokkosis, A.G.; Lamari, F.N.; Margarity, M. Investigation of the neuroprotective action of saffron (Crocus sativus L.) in aluminum-exposed adult mice through behavioral and neurobiochemical assessment. *Food Chem Toxicol* **2013**, *52*, 163-170, https://doi.org/10.1016/j.fct.2012.11.016.
124. Naghibi, S.M.; Hosseini, M.; Khani, F.; Rahimi, M.; Vafaee, F.; Rakhshandeh, H.; Aghaie, A. Effect of aqueous extract of crocus sativus L. on morphine-induced memory impairment. *Adv Pharmacol Sci* **2012**, *2012*, https://doi.org/10.1155/2012/494367.
125. Khan, M.B.; Hoda, M.N.; Ishrat, T.; Ahmad, S.; Khan, M.M.; Ahmad, A.; Yusuf, S.; Islam, F. Neuroprotective efficacy of Nardostachys jatamansi and crocetin in conjunction with selenium in cognitive impairment. *Neurol Sci* **2012**, *33*, 1011-1020, https://doi.org/10.1007/s10072-011-0880-1.
126. Hosseinzadeh, H.; Sadeghnia, H.R.; Ghaeni, F.A.; Motamedshariaty, V.S.; Mohajeri, S.A. Effects of saffron (Crocus sativus L.) and its active constituent, crocin, on recognition and spatial memory after chronic cerebral hypoperfusion in rats. *Phytother Res* **2012**, *26*, 381-386, https://doi.org/10.1002/ptr.3566.
127. Dashti-r, M.H.; Zeinali, F.; Anvari, M.; Hosseini, S.M. Saffron (Crocus sativus L.) extract prevents and improves D-galactose and NaNO2 induced memory impairment in mice. *EXCLI Journal* **2012**, *11*, 328-337.
128. Cong, W.H.; Yang, B.; Xu, L.; Dong, X.X.; Sheng, L.S.; Hou, J.C.; Liu, J.X. Herbal Extracts Combination (WNK) Prevents Decline in Spatial Learning and Memory in APP/PS1 Mice through Improvement of Hippocampal A beta Plaque Formation, Histopathology, and Ultrastructure. *Evid Based Complement Alternat Med* **2012**, https://doi.org/10.1155/2012/478190.
129. Shati, A.A.; Elsaid, F.G.; Hafez, E.E. Biochemical and molecular aspects of aluminium chloride-induced neurotoxicity in mice and the protective role of Crocus sativus L. extraction and honey syrup. *Neuroscience* **2011**, *175*, 66-74, https://doi.org/10.1016/j.neuroscience.2010.11.043.
130. Papandreou, M.A.; Tsachaki, M.; Efthimiopoulos, S.; Cordopatis, P.; Lamari, F.N.; Margarity, M. Memory enhancing effects of saffron in aged mice are correlated with antioxidant protection. *Behav Brain Res* **2011**, *219*, 197-204, https://doi.org/10.1016/j.bbr.2011.01.007.

131. Ghadrdoost, B.; Vafaei, A.A.; Rashidy-Pour, A.; Hajisoltani, R.; Bandegi, A.R.; Motamedi, F.; Haghighi, S.; Sameni, H.R.; Pahlvan, S. Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. *Eur J Pharmacol* **2011**, *667*, 222-229, https://doi.org/10.1016/j.ejphar.2011.05.012.
132. Khalili, M.; Kiasalari, Z.; Rahmati, B.; Narenjkar, J. Behavioral and Histological Analysis of Crocus Sativus Effect in Intracerebroventricular Streptozotocin Model of Alzheimer Disease in Rats. *Iran J Pathol* **2010**, *5*.
133. Khalili, M.; Hamzeh, F. Effects of active constituents of crocus sativus L, crocin on streptozocin-induced model of sporadic Alzheimer's disease in male rats. *Iran Biomed J* **2010**, *14*, 59-65.
134. Khalili, M.; Roghani, M.; Ekhlasi, M. The effect of aqueous crocus sativus L. extract on intracerebroventricular streptozotocin-induced cognitive deficits in rat: a behavioral analysis. *Iran J Pharm Res* **2009**, 8, 185-191.
135. Pitsikas, N.; Sakellaridis, N. Crocus sativus L. extracts antagonize memory impairments in different behavioural tasks in the rat. *Behav Brain Res* **2006**, *173*, 112-115, https://doi.org/10.1016/j.bbr.2006.06.005.
136. Abe, K.; Sugiura, M.; Yamaguchi, S.; Shoyama, Y.; Saito, H. Saffron extract prevents acetaldehyde-induced inhibition of long-term potentiation in the rat dentate gyrus in vivo. *Brain Res* **1999**, *851*, 287-289, https://doi.org/10.1016/S0006-8993(99)02174-5.
137. Sugiura, M.; Saito, H.; Nishiyama, N.; Shoyama, Y. Crocin Improves the Ethanol-induced Impairment of Learning Behaviors of Mice in Passive Avoidance Tasks. *Proc Jpn Acad Ser B Phys Biol Sci* **1995**, *71*, 319-324, https://doi.org/10.2183/pjab.71.319.
138. Rahmani, S.; Saberzadeh, J.; Takhshid, M.A. The Hydroalcoholic Extract of Saffron Protects PC12 Cells against Aluminum-Induced Cell Death and Oxidative Stress in Vitro. *Iran J Med Sci* **2020**, *45*, 59-66, https://doi.org/10.30476/ijms.2019.44971.
139. Rafieipour, F.; Hadipour, E.; Emami, S.A.; Asili, J.; Tayarani-Najaran, Z. Safranal protects against beta-amyloid peptide-induced cell toxicity in PC12 cells via MAPK and PI3 K pathways. *Metabolic Brain Disease* **2019**, *34*, 165-172, https://doi.org/10.1007/s11011-018-0329-9.
140. Patil, D.N.; Patil, S.A.; Sistla, S.; Jadhav, J.P. Comparative biophysical characterization: A screening tool for acetylcholinesterase inhibitors. *PLoS ONE* **2019**, *14*, https://doi.org/10.1371/journal.pone.0215291.
141. Chalatsa, I.; Arvanitis, D.A.; Koulakiotis, N.S.; Giagini, A.; Skaltsounis, A.L.; Papadopoulou-Daifoti, Z.; Tsarbopoulos, A.; Sanoudou, D. The Crocus sativus Compounds trans-Crocin 4 and trans-Crocetin Modulate the Amyloidogenic Pathway and Tau Misprocessing in Alzheimer Disease Neuronal Cell Culture Models. *Front Neurosci* **2019**, *13*, https://doi.org/10.3389/fnins.2019.00249.
142. Inoue, E.; Shimizu, Y.; Masui, R.; Hayakawa, T.; Tsubonoya, T.; Hori, S.; Sudoh, K. Effects of saffron and its constituents, crocin-1, crocin-2, and crocetin on alpha-synuclein fibrils. *J Nat Med* **2018**, *72*, 274-279, https://doi.org/10.1007/s11418-017-1150-1.
143. Tiribuzi, R.; Crispoltoni, L.; Chiurchiù, V.; Casella, A.; Montecchiani, C.; Del Pino, A.M.; Maccarrone, M.; Palmerini, C.A.; Caltagirone, C.; Kawarai, T.; Orlacchio, A.; Orlacchio, A.Trans-crocetin improves amyloid-beta degradation in monocytes from Alzheimer's Disease patients. *J Neurol Sci* **2017**, *372*, 408-412, https://doi.org/10.1016/j.jns.2016.11.004.
144. Seto, S.W.; Chang, D.; Ko, W.M.; Zhou, X.; Kiat, H.; Bensoussan, A.; Lee, S.M.; Hoi, M.P.; Steiner, G.Z.; Liu, J. Sailuotong Prevents Hydrogen Peroxide (H(2)O(2))-Induced Injury in EA.hy926 Cells. *Int J Mol Sci* **2017**, *18*, https://doi.org/10.3390/ijms18010095.
145. Amin, H.; Nieus, T.; Lonardoni, D.; Maccione, A.; Berdondini, L. High-resolution bioelectrical imaging of Aβ-induced network dysfunction on CMOS-MEAs for neurotoxicity and rescue studies. *Scientific reports* **2017**, *7*, https://doi.org/10.1038/s41598-017-02635-x.
146. Morelli, S.; Salerno, S.; Piscioneri, A.; Tasselli, F.; Drioli, E.; De Bartolo, L. Neuronal membrane bioreactor as a tool for testing crocin neuroprotective effect in Alzheimer's disease. *Chem Eng J* **2016**, *305*, 69-78, https://doi.org/10.1016/j.cej.2016.01.035.
147. Matsumura, S.; Murata, K.; Yoshioka, Y.; Matsuda, H. Search for β-Secretase Inhibitors from Natural Spices. *Nat Prod Commun* **2016**, *11*, 507-510.
148. Nanasombat, S.; Thonglong, J.; Jitlakha, J. Formulation and characterization of novel functional beverages with antioxidant and anti-acetylcholinesterase activities. *Bioact Compd Health Dis* **2015**, *5*, 1-16, https://doi.org/10.31989/ffhd.v5i1.162.
149. Karakani, A.M.; Riazi, G.; Mahmood Ghaffari, S.; Ahmadian, S.; Mokhtari, F.; Jalili Firuzi, M.; Zahra Bathaie, S. Inhibitory effect of corcin on aggregation of 1N/4R human tau protein in vitro. *Iran J Basic Med Sci* **2015**, *18*, 485-492.
150. Yoshino, Y.; Ishisaka, M.; Umigai, N.; Shimazawa, M.; Tsuruma, K.; Hara, H. Crocetin Prevents Amyloid β1-42-Induced Cell Death in Murine Hippocampal Cells. *Pharmacology & Pharmacy* **2014**, *5*, https://doi.org/10.4236/pp.2014.51007.
151. Ghahghaei, A.; Bathaie, S.Z.; Kheirkhah, H.; Bahraminejad, E. The protective effect of crocin on the amyloid fibril formation of aβ42 peptide in vitro. *Cell Mol Biol Lett* **2013**, *18*, 328-339, https://doi.org/10.2478/s11658-013-0092-1.

152. Ghahghaei, A.; Bathaie, S.Z.; Bahraminejad, E. Mechanisms of the Effects of Crocin on Aggregation and Deposition of A beta 1-40 Fibrils in Alzheimer's Disease. *Int J Pept Res Ther* **2012**, *18*, 347-351, https://doi.org/10.1007/s10989-012-9308-x.
153. Geromichalos, G.D.; Lamari, F.N.; Papandreou, M.A.; Trafalis, D.T.; Margarity, M.; Papageorgiou, A.; Sinakos, Z. Saffron as a source of novel acetylcholinesterase inhibitors: Molecular docking and in vitro enzymatic studies. *J Agric Food Chem* **2012**, *60*, 6131-6138, https://doi.org/10.1021/jf300589c.
154. Ahn, J.H.; Hu, Y.; Hernandez, M.; Kim, J.R. Crocetin inhibits beta-amyloid fibrillization and stabilizes beta-amyloid oligomers. *Biochem Biophys Res Commun* **2011**, *414*, 79-83, https://doi.org/10.1016/j.bbrc.2011.09.025.
155. Ebrahim-Habibi, M.B.; Amininasab, M.; Ebrahim-Habibi, A.; Sabbaghian, M.; Nemat-Gorgani, M. Fibrillation of α-lactalbumin: Effect of crocin and safranal, two natural small molecules from Crocus sativus. *Biopolymers* **2010**, *93*, 854-865, https://doi.org/10.1002/bip.21477.
156. Nam, K.N.; Park, Y.M.; Jung, H.J.; Lee, J.Y.; Min, B.D.; Park, S.U.; Jung, W.S.; Cho, K.H.; Park, J.H.; Kang, I.; Hong, J.W.; Lee, E.H. Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells. *Eur J Pharmacol* **2010**, *648*, 110-116, https://doi.org/10.1016/j.ejphar.2010.09.003.
157. Gholamhoseinian, A.; Moradi, M.N.; Sharifi-far, F. Screening the methanol extracts of some Iranian plants for acetylcholinesterase inhibitory activity. *Res Pharm Sci* **2009**, *4*, 105-112.
158. Papandreou, M.A.; Kanakis, C.D.; Polissiou, M.G.; Efthimiopoulos, S.; Cordopatis, P.; Margarity, M.; Lamari, F.N. Inhibitory activity on amyloid-beta aggregation and antioxidant properties of Crocus sativus stigmas extract and its crocin constituents. *J Agric Food Chem* **2006**, *54*, 8762-8768, https://doi.org/10.1021/jf061932a.
159. Soeda, S.; Ochiai, T.; Paopong, L.; Tanaka, H.; Shoyama, Y.; Shimeno, H. Crocin suppresses tumor necrosis factor-α-induced cell death of neuronally differentiated PC-12 cells. *Life Sci* **2001**, *69*, 2887-2898, https://doi.org/10.1016/S0024-3205(01)01357-1.
160. Abe, K.; Sugiura, M.; Shoyama, Y.; Saito, H. Crocin antagonizes ethanol inhibition of NMDA receptor-mediated responses in rat hippocampal neurons. *Brain Res* **1998**, *787*, 132-138, https://doi.org/10.1016/S0006-8993(97)01505-9.
161. Sugiura, M.; Shoyama, Y.; Saito, H.; Abe, K. Crocin (crocetin di-gentiobiose ester) prevents the inhibitory effect of ethanol on long-term potentiation in the dentate gyrus in vivo. *J Pharmacol Exp Ther* **1994**, *271*, 703-707.
162. Moazen-Zadeh, E.; Abbasi, S.H.; Safi-Aghdam, H.; Shahmansouri, N.; Arjmandi-Beglar, A.; Hajhosseinn Talasaz, A.; Salehiomran, A.; Forghani, S.; Akhondzadeh, S. Effects of Saffron on Cognition, Anxiety, and Depression in Patients Undergoing Coronary Artery Bypass Grafting: A Randomized Double-Blind Placebo-Controlled Trial. *J Altern Complement Med* **2018**, *24*, 361-368, https://doi.org/10.1089/acm.2017.0173.
163. Akouchekian, S.; Omranifard, V.; Maracy, M.; Pedram, A.; Zefreh, A. Efficacy of herbal combination of sedge, saffron, and Astragalus honey on major neurocognitive disorder. *J Res Med Sci* **2018**, *23*, https://doi.org/10.4103/jrms.JRMS_949_17.
164. Cicero, A.F.; Bove, M.; Colletti, A.; Rizzo, M.; Fogacci, F.; Giovannini, M.; Borghi, C. Short-Term Impact of a Combined Nutraceutical on Cognitive Function, Perceived Stress and Depression in Young Elderly with Cognitive Impairment: A Pilot, Double-Blind, Randomized Clinical Trial. *J Prev Alzheimers Dis* **2017**, *4*, 12-15.
165. Tsolaki, M.; Karathanasi, E.; Lazarou, I.; Dovas, K.; Verykouki, E.; Karakostas, A.; Georgiadis, K.; Tsolaki, A.; Adam, K.; Kompatsiaris, I.; Sinakos, Z. Efficacy and safety of crocus sativus L. in patients with mild cognitive impairment: One year single-blind randomized, with parallel groups, clinical trial. *J Alzheimers Dis* **2016**, *54*, 129-133, https://doi.org/10.3233/JAD-160304.
166. Liang, J.H.; Li, F.; Wei, C.B.; Song, H.Q.; Wu, L.Y.; Tang, Y.; Jia, J.P. Rationale and Design of a Multicenter, Phase 2 Clinical Trial to Investigate the Efficacy of Traditional Chinese Medicine SaiLuoTong in Vascular Dementia. *J Stroke Cerebrovasc Dis* **2014**, *23*, 2626-2634, https://doi.org/10.1016/j.jstrokecerebrovasdis.2014.06.005.
167. Farokhnia, M.; Sabet, M.S.; Iranpour, N.; Gougol, A.; Yekehtaz, H.; Alimardani, R.; Farsad, F.; Kamalipour, M.; Akhondzadeh, S. Comparing the efficacy and safety of Crocus sativus L. with memantine in patients with moderate to severe Alzheimer's disease: a double-blind randomized clinical trial. *Hum Psychopharmacol* **2014**, *29*, 351-359, https://doi.org/10.1002/hup.2412.
168. Akhondzadeh, S.; Shafiee Sabet, M.; Harirchian, M.H.; Togha, M.; Cheraghmakani, H.; Razeghi, S.; Hejazi, S.S.; Yousefi, M.H.; Alimardani, R.; Jamshidi, A.; Rezazadeh, S.-A.; Yousefi, A.; Zare, F.; Moradi, A.; Vossoughi, A. A A 22-week, multicenter, randomized, double-blind controlled trial of Crocus sativus in the treatment of mild-to-moderate Alzheimer's disease. *Psychopharmacology* **2010**, *207*, 637-643, https://doi.org/10.1007/s00213-009-1706-1.
169. Akhondzadeh, S.; Shafiee Sabet, M.; Harirchian, M.H.; Togha, M.; Cheraghmakani, H.; Razeghi, S.; Hejazi, S.S.; Yousefi, M.H.; Alimardani, R.; Jamshidi, A.; Rezazadeh, S.A.; Yousefi, A.; Zare, F.; Moradi, A.; Vossoughi, A. Saffron in the treatment of patients with mild to moderate Alzheimer's disease: a 16-week, randomized and placebo-controlled trial. *J Clin Pharm Ther* **2010**, *35*, 581-588, https://doi.org/10.1111/j.1365-2710.2009.01133.x.

170. Moshiri, M.; Vahabzadeh, M.; Hosseinzadeh, H. Clinical applications of saffron (Crocus sativus) and its constituents: a review. *Drug Res* **2015**, *65*, 287-295, https://doi.org/10.1055/s-0034-1375681.
171. Mohamadpour, A.; Ayati, Z.; Parizadeh, M.R.; Rajbai, O.; Hosseinzadeh, H. Safety Evaluation of Crocin (a constituent of saffron) Tablets in Healthy Volunteers. *Iran J Basic Med Sci* **2013**, *16*, 39-46.
172. Hosseini, A.; Razavi, B.M.; Hosseinzadeh, H. Pharmacokinetic properties of saffron and its active components. *Eur J Drug Metab Pharmacokinet* **2018**, *43*, 383-390, https://doi.org/10.1007/s13318-017-0449-3.
173. Moratalla-López, N.; Bagur, M.J.; Lorenzo, C.; Martínez-Navarro, M.; Salinas, M.R.; Alonso, G.L. Bioactivity and bioavailability of the major metabolites of Crocus sativus L. Flower. *Molecules* **2019**, *24*, 2827, https://doi.org/10.3390/molecules24152827.
174. Rahaiee, S.; Shojaosadati, S.A.; Hashemi, M.; Moini, S.; Razavi, S.H. Improvement of crocin stability by biodegradeble nanoparticles of chitosan-alginate. *Int J Biol Macromol* **2015**, *79*, 423-432, https://doi.org/10.1016/j.ijbiomac.2015.04.041.