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SYSTEMATIC REVIEW ARTICLE

Crocus Sativus L. (Saffron) in Alzheimer's Disease Treatment: Bioactive Effects on Cognitive Impairment

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Abstract: *Crocus sativus* L. (saffron) appears to own neuroprotective effects on cognitive impairment in patients with Alzheimer's disease (AD). The purpose of this work is to review evidence and mechanisms of saffron-induced therapeutic outcomes and measureable cognitive benefits in AD.

The literature was reviewed, and preclinical and clinical studies were identified. *In vitro* and *in vivo* preclinical studies were selected according to these criteria: 1) development of saffron pharmacological profile on biological or biophysical endpoints; 2) evaluation of saffron efficacy using animal screens as an AD model, and 3) duration of the studies of at least 3 months. As for the clinical studies, the selection criteria included: 1) patients aged ≥ 60, 2) AD diagnosis according to National Institute on Aging-Alzheimer's Association (NIAAA) criteria, and 3) appropriate procedures to assess cognitive, functional, and clinical status. A total of 1477 studies published until November 2020 were identified during an initial phase, of which 24 met the inclusion criteria and were selected for this review.

Seventeen *in vitro* and *in vivo* preclinical studies have described the efficacy of saffron on cognitive impairment in animal models of AD, highlighting that crocin appears to be able to regulate glutamate levels, reduce oxidative stress, and modulate Aβ and *tau* protein aggregation. Only four clinical studies have indicated that the effects of saffron on cognitive impairment were not different from those produced by donepezil and memantine and that it had a better safety profile.

Saffron and its compounds should be further investigated in order to consider them a safer alternative in AD treatment.

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1. INTRODUCTION

Worldwide, Alzheimer's disease (AD) is the major prevalent neurodegenerative disease related to aging, accounting for 60-70% of overall dementia cases [1]. Currently, the available treatments for AD are only supportive, namely the following four: memantine (a glutamate receptor antagonist), donepezil, rivastigmine and galantamine, which are acetylcholinesterase (AChE) inhibitors. These drugs seem to provide a limited symptomatic improvement in the cognitive impairment and neuropsychiatric symptoms linked to AD, but none of them can provide a real disease-modifying effect.

AD symptomatic changes are associated with reduced acetylcholine production, excessive glutamate release [2], deposition of extracellular amyloid-β (Aβ) peptide as plaques in brain tissue and blood vessel [3, 4] and neurofibrillary tangles (intracellular aggregation of hyperphosphorylated tau protein) [5, 6].

Based on the aforesaid assumptions, AD-modifying therapeutic approaches need to be developed in order to slow down AD pathology, paying particular attention to the natural compounds that are able to exhibit enhanced cognition-improving effects.

Currently, several researchers have studied extensively *Crocus sativus* L. (blue purple saffron flower) extracts for memory and brain health. *Crocus sativus* is native to Middle East and is a member of Iridaceae (iris) family. Its name derives from an Arab word for yellow, which reflects the high

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carotenoid pigment concentration in saffron flowers' stigmas [7].

Considering that the chemical components can vary according to the environmental conditions in the country of origin, saffron's chemical composition is made of around 63% sugars (comprising starch, gums, reducing sugars, dextrins, pectin, and pentosans), 12% protein, 10% moisture, 5% crude fibre, 5% minerals, 5% fat [8] and traces of riboflavin and thiamine vitamins [9].

Saffron's sensory profile (color, aroma, and taste) and its health-promoting properties mainly come from its major bioactive compounds, which are the following: 1) crocin (mono-glycosyl/di-glycosyl polyene esters), 2) crocetin (carotenoid dicarboxylic acid precursor of crocin), 3) safranal (product of the carotenoid zeaxanthin degradation) and 4) picrocrocin (monoterpene glycoside precursor of safranal) [9-14]. All chemical structures are collected in Fig. (**1a**), Fig. (**1b**), Fig. (**1c**) and Fig. (**1d**).

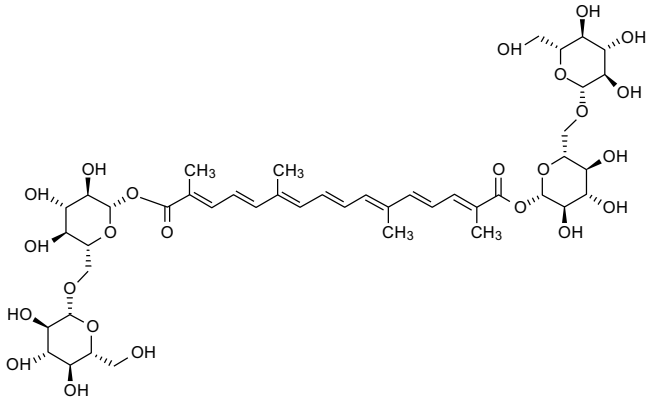

Fig. (1a). Chemical structures of crocin.

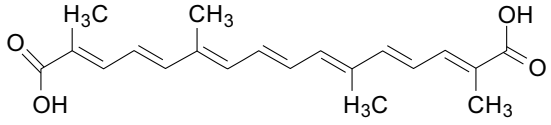

Fig. (1b). Chemical structures of crocetin.

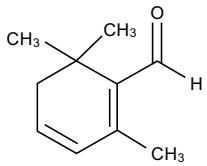

Fig. (1c). Chemical structures of safranal.

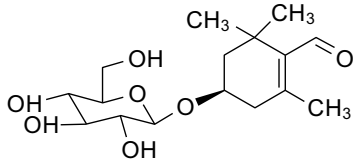

Fig. (1d). Chemical structures of picrocrocin.

Characteristically red in color, crocin {[(2S, 3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxymethyl]oxan-2-yl] (2E,4E,6E,8E,10E,12E,14E)-2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptaenedioate} constitutes approximately 25- 35% of saffron's total dry matter [15]. It rapidly melts in water to become an orange liquid and for this reason is extensively used as a natural colorant for food [16, 17]. The major copious crocin with a high solubility is crocin 1 (or α-crocin), which is a digentiobioside.

Central core of crocin consists in the crocetin chemical structure [(2E,4E,6E,8E,10E,12E,14E)-2,6,11,15-Tetramethyl-2,4,6,8,10,12,14-hexadecaheptaenedioic acid]. Crocetin is a natural apocarotenoid dicarboxylic acid and is responsible for color of saffron.

Another important compound is safranal (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde), a degradation product of the carotenoid zeaxanthin *via* picrocrocin deglycosylation. Safranal is a constituent of saffron and the main reason for its aroma, representing about 0.001% to 0.006% of the dry matter [18, 19].

Picrocrocin [C16H26O7, 4-(-D-glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carbox-aldehyde] constitutes around 26% of saffron's dry matter [20] and is second most abundant component in terms of weight [8]. Saffron derives its taste from picrocrocin that is the precursor of safranal and is most stable than crocetin esters. Picrocrocin degradation kinetics in aqueous saffron extracts upon thermal treatment from 5°C to 70°C was investigated [21].

Main carotenoids in saffron are glycosidic esters and therefore soluble in water. The mechanisms of actions of *Crocus sativus* extract are shown in Fig. (**2**). From a pharmacokinetic point of view, crocins are not immediately assimilated after oral administration but are mainly hydrolyzed to crocetin in intestinal tract [22]. Crocetin is absorbed more rapidly compared to other carotenoids and, after one hour from administration (peaking around 4 h later), it is identifiable in plasma [23]. Kyriakoudi *et al*. have shown that approximately 50% of crocetin and 70% of picrocrocin were bio-accessible under *in vitro* gastrointestinal and digestion conditions [24].

Lautenschlager *et al*. have reported that probably, after oral consumption, the crocins are not bioavailable in saffron systemic compartment [25]. Crocins are rapidly hydrolyzed to deglycosylated trans-crocetin (assimilated by passive diffusion *via* the intestinal mucosa layer) by intestinal epithelium enzymes and intestinal microbiota [22]. It seems that trans-crocetin is unique active metabolite able to cross blood- brain barrier (BBB) and influence central nervous system (CNS) [15]. Trans-crocetin pharmacokinetics was tested and validated in animal models [26-28] and healthy people's plasma [29].

Trans-Crocetin is smaller and most hydrophilic compared to other carotenoids (*e.g.*, carotene, lycopene and lutein), which take more time to reach peak concentrations [26].

Asai *et al*. have shown that crocin is hydrolyzed to trans-crocetin and cis-crocetin immediately after oral administration [20]. Thus, in liver and/or in intestinal mucosa, trans-crocetin can be partially combined with mono and di-glucuronides and reach the bloodstream through the portal vein [23].

Numerous studies have considered saffron for its anti-cancer [30-40] and anti-atherosclerosis properties [27, 41-43]. It prevents gastric ulcers [44-46], insulin resistance [47, 48] and enhances digestion [49]. Saffron also produces anti-oxidative [50-53], anti-inflammatory [54], anti-convulsion [54], anti-nociceptive [54], anti-depressive [55-60] and anxiolytic [61, 62] effects. In addition to these properties, saffron has neuroprotective effects [51, 63, 64] and all the studies have shown that it slightly enhances cognitive function in patients with AD [65, 66].

The purpose of this study was to review evidence and mechanisms through which *Crocus sativus L*. (saffron) induces therapeutic outcomes and measurable cognitive improvements in AD.

2. MATERIALS AND METHODS

Literature searching was run by Scopus, PubMed, ScienceDirect, Embase and CINAHL until November 2020. The PRISMA guidelines for systematic review were followed [67], and Standards for the Reporting of Diagnostic accuracy studies in dementia (STARDdem) [68] was employed to evaluate quality of study.

The search queries included the following terms (*Crocus sativus L.* OR *Crocus* OR saffron OR crocin OR crocetin OR safranal OR picrocrocin) combined with terms to determine the outcomes of interest (Alzheimer's disease AND [treatment OR effect AND neuroprotective OR AChE inhibitor OR antioxidant OR anti-A*β* OR *tau* protein inhibitor] OR memory OR learning).

A reviewer scanned all abstracts recovered by electronic search and identified the articles according to inclusion criteria deserving to be extensively reviewed.

The inclusion criteria for the *in vitro* and *in vivo* preclinical studies included: 1) development of saffron pharmacological profile on biological or biophysical endpoints; 2) evaluation of saffron efficacy using animal screens as an AD model, and 3) studies conducted for at least 3 months. The selection criteria for the clinical studies included research articles with 1) age ≥ 60 years; 2) diagnosis of AD according to National Institute on Aging-Alzheimer's Association (NIAAA) criteria [69], and 3) appropriate procedures to assess cognitive, functional and clinical status. The exclusion criteria were the following: 1) no English editing, 2) studies conducted < 3 months, 3) age < 60 years, 4) diagnosis of non-AD dementia, and 5) no appropriate clinical and cognitive evaluation.

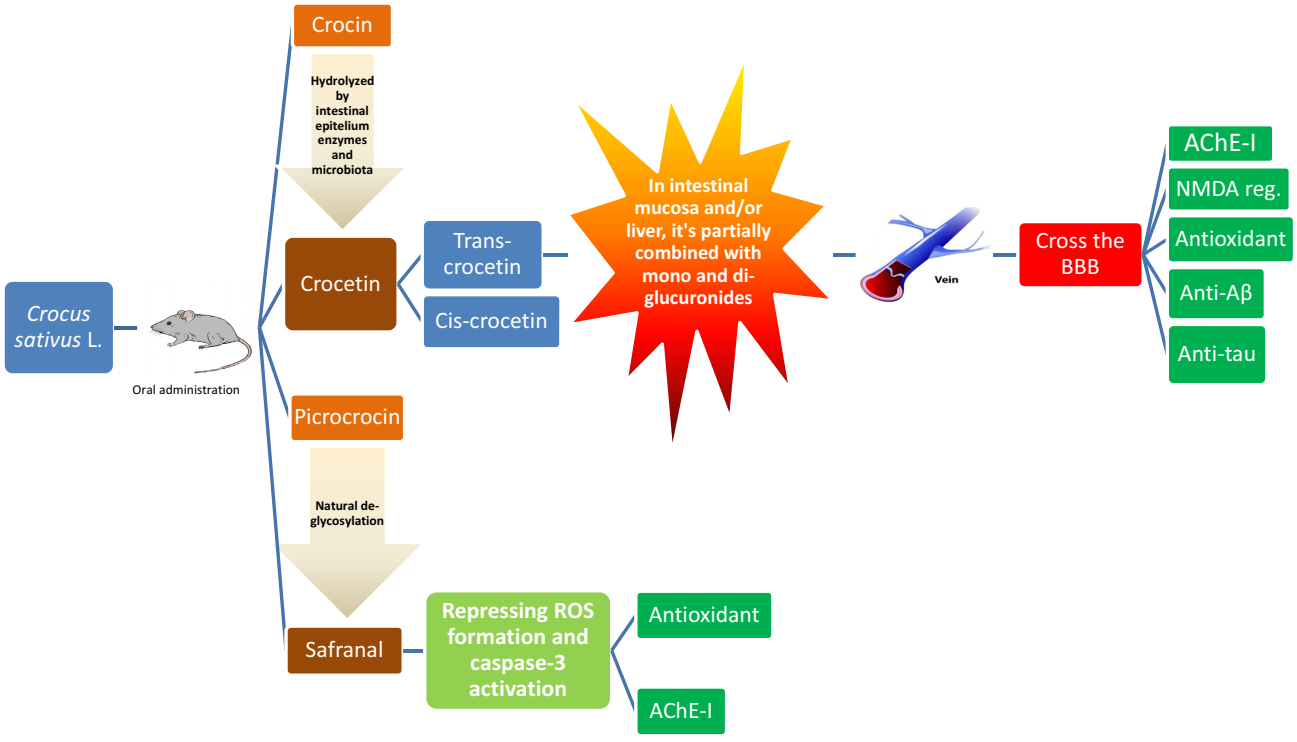

Fig. (2). Mechanisms of action of saffron extracts for the treatment of Alzheimer's disease from preclinical studies.
ROS, reactive oxygen species; **AChE-I**, acetylcholinesterase inhibitors; **BBB**, blood–brain barrier; **NMDA**, N-methyl-D-aspartate receptor; **Anti-A*β***, anti-beta amyloid. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

As shown in Fig. (**3**), a total of 1477 articles were identified, of which 498 duplicates were deleted. After abstract evaluation, 870 studies were kept out. Another 85 studies were kept out after a more comprehensive examination. Consequently, 24 published studies were suitable for this systematic review.

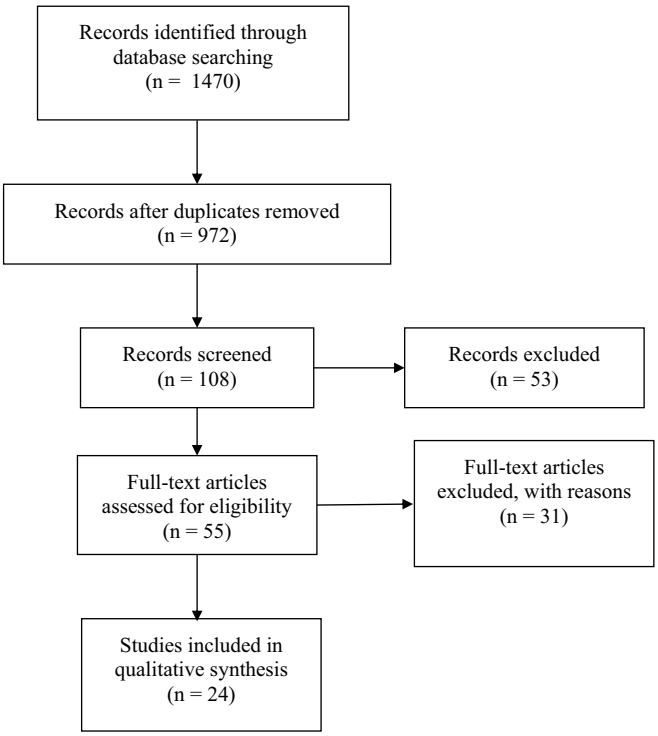

Fig. (3). Flow diagram outlining the selection procedure to identify articles which were included in the analysis of *Crocus sativus* L. (saffron) in AD treatment.

An inductive approach was applied to the analysis [70]. The results section was divided into three categories:

- *In vitro* and *in vivo* preclinical studies
- Clinical studies
- Toxicity evaluation

Co-authors supplied a complete summary and accurate organization of all studies extracting transparency, approach, methodology, and strengths/weaknesses [71, 72].

3. RESULTS AND DISCUSSION

3.1. *In Vitro* and *in Vivo* Preclinical Studies

The preclinical literature regarding the effects of *Crocus sativus* constituents on AD is provided in Table **1** and divided into three subsections (Effects on brain acetylcholinesterase activity and glutamate release, Effects on oxidative distress and brain cells/tissues, and Effects on Aβ peptide and *tau* protein) as shown below.

3.1.1. Effects on Brain Acetylcholinesterase Activity and Glutamate Release

Only one study has reported that aqueous and methanolic saffron extract presented a moderate activity as AChE inhibitor (up to 30%), showing that safranal interplays uniquely with AChE binding site, whereas crocetin and dimethylcrocetin simultaneously bind to peripheral and catalytic anionic sites [73].

Three studies, instead, have been carried out to observe the effects of saffron components on N-metil-D-aspartato (NMDA) activity, which plays a significant role in brain glutamate levels regulating. Hypoactivity of NMDA receptor damages neuronal plasticity and its hyperactivity provokes an excessive production of glutamate with consequent brain cell death and neuronal damage [2]. Based on these assumptions, a study has reported that the cytoprotective activity of saffron extracts and crocetin had a high affinity at phencyclidine binding NMDA receptor site and sigma(1) receptor, while crocins and picrocrocin were not active [74].

In vitro and *in vivo*, Abe *et al*. have previously found that crocin antagonizes inhibitory effect of ethanol in rat hippocampus on long-term potentiation [75]. The aforesaid researchers have suggested that crocin precisely antagonizes inhibitory effect of ethanol on NMDA receptor-mediated responses in dentate gyrus of rat hippocampal slices [75]. In another study, an oral pre-administration of 50-200 mg/kg of crocin enhanced memory impairment in ethanol-treated rats in a dose-dependent manner [76]. In above-mentioned studies, the effects of saffron on long-term potentiation (persistent strengthening of synaptic plasticity that underlies memory and learning) have been widely studied, as highlighted in three systematic reviews [52, 77, 78].

3.1.2. Effects on Oxidative Stress

The oxidative stress degree is considered a risk factor for AD progression [79], which is characterized by brain cell death and synaptic activity loss.

It is generally thought that streptozocin (STZ)-induced neurotoxicity does not contribute to changes in functional NMDA response and is associated with free radical generation causing cognitive impairment. A study has demonstrated that the treatment with crocin (30 mg/kg) for 3 weeks significantly improved cognitive impairment caused by intracerebroventricular injection of STZ, which induced a model of sporadic AD in male rats [80]. Another study has shown that crocin administration (100 mg/kg, p.o., for 21 consecutive days, starting 1 h prior to STZ first dose) improved cognitive tasks and produced a significant decrease of malondialdehyde (MDA) levels and increase of total thiol content and glutathione peroxidase (GPx) activity in STZ-lesioned rats [81].

Considering that chronic aluminum (Al)-induced neurotoxicity model matches better AD and its pathobiological hallmarks [82], a study with adult mice has shown a potential reversal response of saffron *vs*. Al toxicity. Al-treatment plus saffron (60 mg extract/kg/day intraperitoneally for

following 6 days) group did not improve cognitive performance, but it significantly reversed Al-induced changes in monoamine oxidase (MAO) activity along with MDA and glutathione (GSH) levels [83].

Another study compared saffron effect (1–250 μg/mL) with those of crocetin and safranal (1–125 μM) on hydrogen peroxide (H_2O_2), which induces toxicity in human neuroblastoma cells. It was suggested that crocetin is only one and strong antioxidant, providing protection in rescuing cell viability, blocking reactive oxygen species (ROS) production and reducing caspase-3 activation [84].

A recent study has reported that saffron and its active constituent crocin can prevent oxidative stress damage to hippocampus, memory and learning impairments [85]. In this study, rats have received saffron extract (30 mg/kg) or crocin (15-30 mg/kg) over a period of 21 days, and have been undergone chronic stress (6 h/day). Therefore, it resulted a significantly higher MDA levels, a significant higher antioxidant enzyme activity (comprising GPx, superoxide dismutase and glutathione reductase), a significant lower total antioxidant reactivity, and a significant decrease in corticosterone plasma levels [85].

Table 1. Characteristics of the saffron extracts for the treatment of Alzheimer's disease in preclinical studies.

Medicinal Use	Agent	Dose (Rout) or Drug Concentration	Observation	Species	Pharmacological Effects	References
AChE-I	Safranal Crocetin Dimethylcrocetin	96.33 μM 107.1 μM 21.09 μM	*In vitro*	-	Safranal interacted only with the binding site of the AChE, but crocetin and dimethylcrocetin bound simultaneously to the catalytic and peripheral anionic sites	[73]
NMDA regulator	Crocus sativus extract Crocetin Crocin Picrocrocin	1-10 μM 1-10 μM 1-10 μM 1-10 μM	*In vitro*	-	Saffron extracts and crocetin had a high affinity to the phencyclidine binding side of the NMDA receptor and at the sigma(1) receptor, while the crocins and picrocrocin were not effective	[74]
	Crocin Picrocrocin	50–100–200mg/kg (p.o. acute) 50–100–200mg/kg (p.o. acute)	*In vivo*	Mouse	Only crocin reversed ethanol-induced deficits	[75]
	Crocin	50-200 mg/kg (p.o. acute)	*In vivo*	Rat	Tretament improved the memory impairment in ethanol-treated rats in dose-dependent method	[76]
Antioxidant	Crocin	15–30mg/kg (i.p. chronic)	*In vivo*	Rat	Treatment of 30mg/kg for three weeks reversed streptozotocin-induced performance deficits in the Y-maze and passive avoidance task	[80]
	Crocin	100mg/kg (p.o. chronic)	*In vivo*	Rat	Treatment with crocin for 21 days reversed streptozotocin-induced spatial memory deficits	[81]
	Crocus sativus extract	60mg/kg (i.p. chronic)	*In vivo*	Mouse	Ineffective to reverse $AlCl_3$-induced memory deficits	[83]
	Crocus sativus extract	60mg/kg (i.p. chronic)	*In vivo*	Aged mouse	Treatment for 7 days reversed age-related memory deficits	[84]
	Crocus sativus extract Crocin	30mg/kg (i.p. chronic) 15–30mg/kg (i.p. chronic)	*In vivo*	Rat	Treatment for 21 days reversed chronic stress-induced	[85]
	Crocin	5 or 20 mg/kg (i.p. chronic)	*In vivo*	Mouse	Treatment from the 5th week onwards (in total 8 weeks) improved cognition and memory abilities	[86]
Anti-Aβ deposition	*Crocus sativus* extract	30 – 600 μM	*In vitro*	-	Trans-crocin-4 inhibited Aβ fibrillogenesis at lower concentrations than dimethylcrocetin	[51]
	Crocin	15 μg/mL	*In vitro*	-	Crocin decreased the number of fibrils formed and significantly reduced the average fibril length of $A\beta_{40}$	[87]
	Crocin	15.4 μM	*In vitro*	-	Crocin allowed the inhibition of $A\beta_{42}$-mediated amyloid fibril formation, and also the disruption of amyloid aggregates	[88]
	Crocetin	174 μM	*In vitro*	-	Crocetin inhibited Aβ fibril formation, destabilized preformed Aβ fibrils, stabilized Aβ oligomers and prevented their conversion into Aβ fibrils	[89]
	Crocetin	1 - 10 μM	*In vitro*	-	Crocetin at 1 - 10 μM protected HT22 cells against $A\beta_{1-42}$-induced neuronal cell death and decreased ROS production increased by $A\beta_{1-42}$	[90]

(Table 1) contd….

Anti-A*β* deposition	Crocin	1, 10 and 50 μM	*In vitro*	-	Concomitant administration of A*β* peptide and crocin was observed a significant dose-dependent inhibition of apoptosis and ROS production, highlighting the crocin capability to prevent the A*β* peptide aggregation	[91]
	Crocin	10 mg/kg (i.p. chronic)	*In vivo*	Mice	Crocin could exert its protective effect against A*β* pathology, by reducing A*β* brain load, including toxic oligomers, via the higher expression of degradation enzymes and ABCA1	[93]
	Crocin	30 mg/kg (i.p. chronic)	*In vivo*	Rat	Crocin improved spatial memory, passive avoidance deficits, hippocampal LTP reduction and CA1 cells apoptosis	[94]
Anti *tau* protein aggregation	Crocin	0.2, 2, 20, 50, 100, 200, 400 and 600 μg/ml	*In vitro*	-	Crocin inhibited *tau* aggregation with IC50 of 100 μg/ml, and suppressed the formation of tau protein filaments	[95]
	Crocin	50 mg/kg (i.p. chronic)	*In vivo*	Mouse	Crocin attenuated MDA, A*β* and *tau* protein levels by MAPKs signalling pathways	[96]

Abbreviations: AChE-I, acetylcholinesterase inhibitors; **NMDA**, N-methyl-D-aspartate receptor; **$AlCl_3$**, aluminium; **A*β***, amyloid-beta; **ROS**, reactive oxygen species; **ABCA1**, adenosine triphosphate binding cassette subfamily A member 1; **LTP**, long term potentiation; **CA1**, Cornu Ammonis area 1; **MDA**, malondialdehyde; **MAPKs**, modulating mitogen-activated protein kinases.

In a more recent study, the neuroprotective effects of crocin against AD were investigated in l-glutamate (L-Glu)-induced HT22 apoptotic cells and in aluminum trichloride (AlCl3) and d-galactose (d-gal)-induced AD mice [86], from which three fundamental results emerge:

- crocin substantially improved the cognition and memory abilities of the mice as measured by their coordination of movement in an open field test and reduced their escape time in the Morris water maze test compared with untreated mice.
- moreover, the biochemical analysis confirmed that crocin was able to reduce the Aβ1-42 content in the mouse brains, increase the levels of glutathione peroxidase, superoxide dismutase, acetylcholine and choline acetyltransferase, and reduce the levels of ROS and acetylcholinesterase in the serum, cerebral cortex and hypothalamus compared with untreated mice.
- immunohistochemical analysis demonstrated that crocin reduced Aβ1-42 deposition in the hippocampus of the brains of treated mice compared with untreated mice.

3.1.3. Effects on Aβ Peptide and tau Protein

Several *in vitro* studies have been performed on the effect of saffron extract and crocin on A*β* aggregation. Two studies have reported inhibitory effect of saffron on $A\beta_{40}$ fibrillogenesis [51, 87]. Another study has reported that crocin inhibited $A\beta_{42}$-mediated amyloid fibril formation and disrupted amyloid aggregates [88]. Moreover, Ahn *et al*. have shown that crocetin, able to pass through BBB, inhibits fibril A*β* formation, destabilizes pre-formed A*β* fibrils, causes A*β* oligomers stabilization [89].

Another study has demonstrated that crocetin owns a strong neuroprotective effect *vs*. $A\beta_{1\text{-}42}$-induced cytotoxicity in hippocampal cells through oxidative stress reducing [90]. It was shown that crocetin at 1-10 μM protected HT22 cells *vs*. $A\beta_{1\text{-}42}$-induced neuronal cell death and decreased ROS production. In an *in vitro* study, a neuronal membrane bioreactor was used as a model of Aβ-induced toxicity associated with AD to assess the neuroprotective effect of crocin in cells for the first time [91]. It was shown that the concomitant administration of A*β* peptide and crocin induced a significant dose-dependent inhibition of ROS production and apoptosis, highlighting the crocin skill to avoid the A*β* peptide aggregation and following neurotoxicity associated with AD [91], as previously demonstrated by Asadi *et al*. [92].

The exact mechanism of crocin effects is still being discussed, but some hypotheses can be suggested. Batarseh *et al*. (2017) investigated the effect of Crocus sativus extract and crocin on A*β* load and the related toxicity [93]. *In vivo* studies, performed on 5XFAD mice used as AD model, demonstrated that crocin added to mice diet produced effects on BBB function and tightness. This was related a slight decrease in total brain A*β* and a significant reduction in A*β*o, the most toxic and pathogenic form of A*β*. Furthermore, in brain homogenates, crocin significantly improved A*β* expression declining neprilysin (NEP) enzyme and adenosine triphosphate binding cassette subfamily A member 1 (ABCA1), a regulatory protein involved in A*β* clearance. These results show that crocin could employ its protective effect against A*β* pathology, through the reduction of the brain load of A*β*, including toxic oligomers and an increased expression of the degrading enzymes and ABCA1. The effects of crocin on A*β*-induced toxicity was recently confirmed by Hadipour *et al*. (2018), who evaluated crocin effects on memory, long-term potentiation (LTP) and neuronal apoptosis using an *in vivo* AD model based on the bilateral administration of A*β* peptide (1-42) [94]. Crocin daily administered for 12 days (30 mg/kg, i.p.) was able to improve spatial memory, passive avoidance deficits, hippocampal LTP reduction and *Cornu Ammonis* area 1 (CA1) cells apoptosis, further confirming the neuroprotective action of crocin against A*β* toxicity.

In addition to the aforesaid studies, other researchers have examined inhibitory effects of crocin on *tau* protein neurofibrillary tangles in AD.

An *in vitro* study has reported the crocin inhibitory effect on aggregation of recombinant human *tau* protein

Table 2. Characteristics of the saffron extracts for the treatment of Alzheimer's disease in clinical studies.

Study Design	No of Patients	Capsule Contents	Dose (Rout)	Severity of Disease	Pharmacological Effects	References
12-month, Single-blind	35	*Crocus sativus* extracts	125 mg per day (p.o.)	Mild cognitive impairment	Significantly better outcome on cognitive function than control group	[65]
22-week, Double-blind	55	Safranal (0.13–0.15 mg) Crocin (1.65–1.75 mg)	15 mg twice per day (p.o.)	Mild to moderate	Similar efficacy and better safety profile compared to donepezil	[66]
16-week, Double-blind	46	Safranal (0.13–0.15 mg) Crocin (1.65–1.75 mg)	15 mg twice per day (p.o.)	Mild to moderate	Significantly better outcome on cognitive function than placebo	[97]
12-month, Double-blind	68	Crocin (1.65–1.75 mg)	15 mg twice per day (p.o.)	Moderate to severe	Similar efficacy and safety profile compared to memantine	[98]

1N/4R isoform with IC50 of 100 μg/ml, and its ability to suppress *tau* protein filament formations [95]. Another study has examined neuroprotective effects of crocin against the toxic effects of oral administration of 3 mg/kg/day acrolein on rat cerebral cortex [96]. It was shown that the crocin co-administration has meaningfully reduced MDA, A*β* and *tau* protein levels by mitogen-activated protein kinases (MAPKs) signaling pathways modulating [91]. These data demonstrated that crocin could be an adjuvant for neurodegenerative disease treatment.

3.2. Clinical Studies

A snapshot of the clinical literature regarding the effects of *Crocus sativus* on cognition is provided in Table **2**.

First study of phase II was a multicentre, double-blind controlled clinical trial with 55 mild-to-moderate AD patients: the participants were casually allocated to take a capsule of saffron 30mg/day (15 mg twice a day, p.o.) or donepezil 10mg/day (5 mg twice a day, p.o.) for 22 weeks [66]. The outcomes have shown that, at this dose, saffron was effective as donepezil in mild-to-moderate AD treatment after 22 weeks, without the adverse effects and risks that occurred after treatment with donepezil [66].

A successive double-blind placebo-controlled study was realized with 46 mild-to-moderate AD patients [97] who were casually allocated to take a capsule of saffron 30 mg/day (15 mg twice a day) or a capsule of placebo (two capsules a day) for 16 weeks. The results of this study indicated that saffron produces a significant improvement in cognitive performance (ADAS-cog: $F = 4{\cdot}12$, d.f. = 1, $p = 0{\cdot}04$), compared to placebo [97].

In a randomized, double-blind parallel-group study, 68 moderate-to-severe AD patients took saffron extract (30 mg/day) or memantine (20 mg/day) capsules for a period of 12 - months [98]. It was demonstrated that saffron extract capsules administration significantly reduced cognitive decline when compared with memantine. Moreover, no significant differences were detected between the groups in terms of adverse event frequency [98].

Finally, a one–year single-blind randomized clinical trial was carried out in 35 patients with multi-domain and amnesic mild cognitive impairment: 17 patients were casually allocated to take a saffron capsule, and 18 patients were randomly assigned to a waiting list for 12 months [65]. The results reported that saffron-treated patients had improved cognitive performance as assessed by Mini-Mental State Examination scores ($p = 0.015$) and Magnetic Resonance Imaging, Electroencephalogram, and Event-Related Potential, while the control group had deteriorated [65].

3.3. Toxicity Evaluation

Even if *Crocus sativus* L. is widely used as a food additive, it needs a toxicity and safety assessment when administrated as medication.

In the clinical trial studies about the *Crocus sativus* L. efficacy in AD and depression, 15–30 mg/day of capsule were prescribed and several minor adverse effects (dry mouth, dizziness, nausea, and vomiting) have been described [57, 59, 66, 98].

A recent acute oral toxicity study showed that IIIM-141 is safe up to the dose of 2000 mg/kg, with no effect on the body weight and on the biochemical/hematological parameters of the rats. The repeated oral administration of IIIM-141 for 28 days at 100 mg/kg dose did not cause any preterminal deaths and abnormalities in Wistar rats [99].

We could not find any study on the interactions between different drugs and saffron in AD.

CONCLUSION

Currently, there is no trustworthy pharmacological treatment for patients with AD. Several natural compounds and their specific molecular targets appear to slow down the onset of AD, delay the progression of disease, and allow for recovery targeting multiple pathological causes through anticholinergic, anti-inflammatory and antioxidant features and without adverse events.

This review provides evidence for the use of saffron and its compounds in AD treatment, considering preclinical and clinical studies.

In vitro and *in vivo* preclinical studies demonstrated efficacy of saffron in attenuating cognitive impairment in animal models of AD. In particular, crocin appears to be multifunctional in brain cell-protection because it is capable of regulating glutamate levels, reducing oxidative stress, modulating A*β* and *tau* protein aggregation and improving cognitive impairment.

However, the number of clinical studies is limited to four trials. Few patients were involved in these studies and a narrow range of cognitive disorders was considered. The results indicated that effects of saffron on cognition were not different from those expressed by donepezil and memantine. However, saffron revealed a better safety profile when compared to donepezil and a similar safety profile when compared to memantine.

In the light of these findings, further clinical studies on saffron compounds at different doses, by themselves or in combination with other natural compounds or commercial chemical drugs (including rivastigmine capsule as well as rivastigmine transdermal) should be conducted.

The research on *Crocus sativus* L. effect in AD treatment is interesting, considering that spice could be as effective as the leading AD pharmaceuticals. Undoubtedly, preventing or reversing AD is complex than anyone drug or spice, but the existing results are already promising in view of saffron as a treatment alternative without side effects and with a quality of life improvement.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

The authors have no conflicts of interest, financial or otherwise.

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REFERENCES

[1] World Health Organization. Dementia. **2017**. http://www.who.int/mediacentre/factsheets/fs362/en/

[2] Finley, J.W.; Gao, S. A Perspective on *Crocus sativus* L. (Saffron) Constituent Crocin: A potent water-soluble antioxidant and potential therapy for alzheimer's disease. *J. Agric. Food Chem.,* **2017**, *65*(5), 1005-1020. http://dx.doi.org/10.1021/acs.jafc.6b04398 PMID: 28098452

[3] Selkoe, D.J. Alzheimer's disease: genes, proteins, and therapy. *Physiol. Rev.,* **2001**, *81*(2), 741-766. http://dx.doi.org/10.1152/physrev.2001.81.2.741 PMID: 11274343

[4] Gonzalez-Velasquez, F.J.; Kotarek, J.A.; Moss, M.A. Soluble aggregates of the amyloid-beta protein selectively stimulate permeability in human brain microvascular endothelial monolayers. *J. Neurochem.,* **2008**, *107*(2), 466-477. http://dx.doi.org/10.1111/j.1471-4159.2008.05618.x PMID: 18702666

[5] Nisbet, R.M.; Polanco, J.C.; Ittner, L.M.; Götz, J. Tau aggregation and its interplay with amyloid-β. *Acta Neuropathol.,* **2015**, *129*(2), 207-220. http://dx.doi.org/10.1007/s00401-014-1371-2 PMID: 25492702

[6] Mondragón-Rodríguez, S.; Perry, G.; Zhu, X.; Boehm, J. Amyloid Beta and tau proteins as therapeutic targets for Alzheimer's disease treatment: Rethinking the current strategy. *Int. J. Alzheimers Dis.,* **2012**, *2012*, 630182. http://dx.doi.org/10.1155/2012/630182 PMID: 22482074

[7] Karazhiyan, H.; Salari, R.; Vazirzadeh, B. Effect of storage time on physiochemical and microbial properties of saffron. *J. Agric. Food Technol.,* **2012**, *2*(4), 61-68.

[8] Melnyk, J.P.; Wang, S.; Marcone, M.F. Chemical and biological properties of the world's most expensive spice: Saffron. *Food Res. Int.,* **2010**, *43*, 1981-1989. http://dx.doi.org/10.1016/j.foodres.2010.07.033

[9] Rios, J.L.; Recio, M.C.; Giner, R.M.; Manez, S. A update review of saffron and its active constituents. *Phytother. Res.,* **1996**, *10*, 189-193. http://dx.doi.org/10.1002/(SICI)1099-1573(199605)10:3<189::AID-PTR754>3.0.CO;2-C

[10] Sarfarazi, M.; Jafari, S.M.; Rajabzadeh, G. Extraction optimization of saffron nutraceuticals through response surface methodology. *Food Anal. Methods,* **2015**, *8*, 2273. http://dx.doi.org/10.1007/s12161-014-9995-3

[11] Khazaei, K.M.; Jafari, S.M.; Ghorbani, M.; Kakhki, A.H.; Sarfarazi, M. Optimization of anthocyanin extraction from saffron petals with response surface methodology. *Food Anal. Methods,* **2016**, *9*(7), 1993-2001. http://dx.doi.org/10.1007/s12161-015-0375-4

[12] Rajabi, H.; Ghorbani, M.; Jafari, S.M.; Mahoonak, A.S.; Rajabzadeh, G. Retention of saffron bioactive components by spray drying encapsulation using maltodextrin, gum Arabic and gelatin as wall materials. *Food Hydrocoll.,* **2015**, *51*, 327-337. http://dx.doi.org/10.1016/j.foodhyd.2015.05.033

[13] Tayebeh, S.; Elham, A.; 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. http://dx.doi.org/10.1016/j.tifs.2016.10.010

[14] Mehrnia, M.A.; Jafari, S.M.; Makhmal-Zadeh, B.S.; Maghsoudlou, Y. Crocin loaded nano-emulsions: Factors affecting emulsion properties in spontaneous emulsification. *Int. J. Biol. Macromol.,* **2016**, *84*, 261-267. http://dx.doi.org/10.1016/j.ijbiomac.2015.12.029 PMID: 26708427

[15] Valle García-Rodríguez, M.; Serrano-Díaz, J.; Tarantilis, P.A.; López-Córcoles, H.; Carmona, M.; Alonso, G.L. Determination of saffron quality by high-performance liquid chromatography. *J. Agric. Food Chem.,* **2014**, *62*(32), 8068-8074. http://dx.doi.org/10.1021/jf5019356 PMID: 25075549

[16] Alonso, G.L.; Salinas, M.R.; Garijo, J.; Sanchez-Fernadez, M.A. Composition of crocins and picrocrocin from Spanish saffron (*Crocus sativus* L.). *J. Food Qual.,* **2001**, *24*(3), 219-233. http://dx.doi.org/10.1111/j.1745-4557.2001.tb00604.x

[17] Sampathu, S.R.; Shivashankar, S.; Lewis, Y.S.; Wood, A.B. Saffron (*Crocus sativus* Linn.) — Cultivation, processing, chemistry and standardization. *Crit. Rev. Food Sci. Nutr.,* **1984**, *20*(2), 123-157. http://dx.doi.org/10.1080/10408398409527386

[18] Carmona, M.; Zalacain, A.; Salinas, M.R.; Alonso, G.L. A new approach to saffron aroma. *Crit. Rev. Food Sci. Nutr.,* **2007**, *47*(2), 145-159. http://dx.doi.org/10.1080/10408390600626511 PMID: 17364699

[19] Maggi, L.; Carmona, M.; del Campo, C.P.; Kanakis, C.D.; Anas-

tasaki, E.; Tarantilis, P.A.; Polissiou, M.G.; Alonso, G.L. Worldwide market screening of saffron volatile composition. *J. Sci. Food Agric.,* **2009**, *89*(11), 1950-1954.
http://dx.doi.org/10.1002/jsfa.3679

[20] Sánchez, A.M.; Carmona, M.; del Campo, C.P.; Alonso, G.L. Solid-phase extraction for picrocrocin determination in the quality control of saffron spice (*Crocus sativus* L.). *Food Chem.,* **2009**, *116*, 792-798.
http://dx.doi.org/10.1016/j.foodchem.2009.03.039

[21] Sánchez, A.M.; Carmona, M.; Jarén-Galán, M.; Mosquera, M.I.; Alonso, G.L. Picrocrocin kinetics in aqueous saffron spice extracts (*Crocus sativus* L.) upon thermal treatment. *J. Agric. Food Chem.,* **2011**, *59*(1), 249-255.
http://dx.doi.org/10.1021/jf102828v PMID: 21141822

[22] Xi, L.; Qian, Z.; Du, P.; Fu, J. Pharmacokinetic properties of crocin (crocetin digentiobiose ester) following oral administration in rats. *Phytomedicine,* **2007**, *14*(9), 633-636.
http://dx.doi.org/10.1016/j.phymed.2006.11.028 PMID: 17215113

[23] Bagur, J. M.; Alonso Salinas, G.L.; Jiménez-Monreal, A.M.; Chaouqi, S.; Llorens, S.; Martínez-Tomé, M.; Alonso, G.L. Saffron: An old medicinal plant and a potential novel. *Functional Food Molecules,* **2017**, *23*(1), 30.

[24] Kyriakoudi, A.; Tsimidou, M.Z.; O'Callaghan, Y.C.; Galvin, K.; O'Brien, N.M. Changes in total and individual crocetin esters upon *in vitro* gastrointestinal digestion of saffron aqueous extracts. *J. Agric. Food Chem.,* **2013**, *61*(22), 5318-5327.
http://dx.doi.org/10.1021/jf400540y PMID: 23654200

[25] Lautenschläger, M.; Sendker, J.; Hüwel, S.; Galla, H.J.; Brandt, S.; Düfer, M.; Riehemann, K.; Hensel, A. Intestinal formation of trans-crocetin from saffron extract (*Crocus sativus* L.) and in vitro permeation through intestinal and blood brain barrier. *Phytomedicine,* **2015**, *22*(1), 36-44.
http://dx.doi.org/10.1016/j.phymed.2014.10.009 PMID: 25636868

[26] Asai, A.; Nakano, T.; Takahashi, M.; Nagao, A. Orally administered crocetin and crocins are absorbed into blood plasma as crocetin and its glucuronide conjugates in mice. *J. Agric. Food Chem.,* **2005**, *53*(18), 7302-7306.
http://dx.doi.org/10.1021/jf0509355 PMID: 16131146

[27] Zheng, S.; Qian, Z.; Tang, F.; Sheng, L. Suppression of vascular cell adhesion molecule-1 expression by crocetin contributes to attenuation of atherosclerosis in hypercholesterolemic rabbits. *Biochem. Pharmacol.,* **2005**, *70*(8), 1192-1199.
http://dx.doi.org/10.1016/j.bcp.2005.07.034 PMID: 16154540

[28] He, K.; Si, P.; Wang, H.; Tahir, U.; Chen, K.; Xiao, J.; Duan, X.; Huang, R.; Xiang, G. Crocetin induces apoptosis of BGC-823 human gastric cancer cells. *Mol. Med. Rep.,* **2014**, *9*(2), 521-526.
http://dx.doi.org/10.3892/mmr.2013.1851 PMID: 24337515

[29] Umigai, N.; Murakami, K.; Ulit, M.V.; Antonio, L.S.; Shirotori, M.; Morikawa, H.; Nakano, T. The pharmacokinetic profile of crocetin in healthy adult human volunteers after a single oral administration. *Phytomedicine,* **2011**, *18*(7), 575-578.
http://dx.doi.org/10.1016/j.phymed.2010.10.019 PMID: 21112749

[30] Salomi, M.J.; Nair, S.C.; Panikkar, K.R. Inhibitory effects of Nigella sativa and saffron (*Crocus sativus*) on chemical carcinogenesis in mice. *Nutr. Cancer,* **1991**, *16*(1), 67-72.
http://dx.doi.org/10.1080/01635589109514142 PMID: 1923908

[31] Abdullaev, F.I. Biological effects of saffron. *Biofactors,* **1993**, *4*(2), 83-86.
PMID: 8347278

[32] Escribano, J.; Alonso, G.L.; Coca-Prados, M.; Fernandez, J.A. Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. *Cancer Lett.,* **1996**, *100*(1-2), 23-30.
http://dx.doi.org/10.1016/0304-3835(95)04067-6 PMID: 8620447

[33] Wang, C.J.; Cheng, T.C.; Liu, J.Y.; Chou, F.P.; Kuo, M.L.; Lin, J.K. Inhibition of protein kinase C and proto-oncogene expression by crocetin in NIH/3T3 cells. *Mol. Carcinog.,* **1996**, *17*(4), 235-240.
http://dx.doi.org/10.1002/(SICI)1098-2744(199612)17:4<235::AID-MC7>3.0.CO;2-C PMID: 8989917

[34] García-Olmo, D.C.; Riese, H.H.; Escribano, J.; Ontañón, J.; Fernandez, J.A.; Atiénzar, M.; García-Olmo, D. Effects of long-term treatment of colon adenocarcinoma with crocin, a carotenoid from saffron (*Crocus sativus* L.): an experimental study in the rat. *Nutr. Cancer,* **1999**, *35*(2), 120-126.
http://dx.doi.org/10.1207/S15327914NC352_4 PMID: 10693164

[35] Molnár, J.; Szabó, D.; Pusztai, R.; Mucsi, I.; Berek, L.; Ocsovszki, I.; Kawata, E.; Shoyama, Y. Membrane associated antitumor effects of crocine-, ginsenoside- and cannabinoid derivates. *Anticancer Res.,* **2000**, *20*(2A), 861-867.
PMID: 10810367

[36] Jagadeeswaran, R.; Thirunavukkarasu, C.; Gunasekaran, P.; Ramamurty, N.; Sakthisekaran, D. *In vitro* studies on the selective cytotoxic effect of crocetin and quercetin. *Fitoterapia,* **2000**, *71*(4), 395-399.
http://dx.doi.org/10.1016/S0367-326X(00)00138-6 PMID: 10925010

[37] Chryssanthi, D.G.; Lamari, F.N.; Iatrou, G.; Pylara, A.; Karamanos, N.K.; Cordopatis, P. Inhibition of breast cancer cell proliferation by style constituents of different *Crocus* species. *Anticancer Res.,* **2007**, *27*(1A), 357-362.
PMID: 17352254

[38] Bathaie, S.Z.; Bolhasani, A.; Hoshyar, R.; Ranjbar, B.; Sabouni, F.; Moosavi-Movahedi, A.A. Interaction of saffron carotenoids as anticancer compounds with ctDNA, Oligo (dG.dC)15, and Oligo (dA.dT)15. *DNA Cell Biol.,* **2007**, *26*(8), 533-540.
http://dx.doi.org/10.1089/dna.2007.0598 PMID: 17688404

[39] Tavakkol-Afshari, J.; Brook, A.; Mousavi, S.H. Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines. *Food Chem. Toxicol.,* **2008**, *46*(11), 3443-3447.
http://dx.doi.org/10.1016/j.fct.2008.08.018 PMID: 18790714

[40] Mousavi, S.H.; Tavakkol-Afshari, J.; Brook, A.; Jafari-Anarkooli, I. Role of caspases and Bax protein in saffron-induced apoptosis in MCF-7 cells. *Food Chem. Toxicol.,* **2009**, *47*(8), 1909-1913.
http://dx.doi.org/10.1016/j.fct.2009.05.017 PMID: 19457443

[41] He, S.Y.; Qian, Z.Y.; Tang, F.T.; Wen, N.; Xu, G.L.; Sheng, L. Effect of crocin on experimental atherosclerosis in quails and its mechanisms. *Life Sci.,* **2005**, *77*(8), 907-921.
http://dx.doi.org/10.1016/j.lfs.2005.02.006 PMID: 15964309

[42] Sheng, L.; Qian, Z.; Zheng, S.; Xi, L. Mechanism of hypolipidemic effect of crocin in rats: crocin inhibits pancreatic lipase. *Eur. J. Pharmacol.,* **2006**, *543*(1-3), 116-122.
http://dx.doi.org/10.1016/j.ejphar.2006.05.038 PMID: 16828739

[43] He, S.Y.; Qian, Z.Y.; Wen, N.; Tang, F.T.; Xu, G.L.; Zhou, C.H. Influence of Crocetin on experimental atherosclerosis in hyperlipidamic-diet quails. *Eur. J. Pharmacol.,* **2007**, *554*(2-3), 191-195.
http://dx.doi.org/10.1016/j.ejphar.2006.09.071 PMID: 17109848

[44] Inoue, E.; Shimizu, Y.; Shoji, M.; Tsuchida, H.; Sano, Y.; Ito, C. Pharmacological properties of N-095, a drug containing red ginseng, polygala root, saffron, antelope horn and aloe wood. *Am. J. Chin. Med.,* **2005**, *33*(1), 49-60.
http://dx.doi.org/10.1142/S0192415X05002655 PMID: 15844833

[45] Kianbakht, S.; Mozaffari, K. Effects of saffron and its active constituents, crocin and safranal on prevention of indomethacin induced gastric ulcers in diabetic and nondiabetic rats. *Faslnamah-i Giyahan-i Daruyi,* **2009**, *8*(5), 30-38.

[46] Xu, G.L.; Li, G.; Ma, H.P.; Zhong, H.; Liu, F.; Ao, G.Z. Preventive effect of crocin in inflamed animals and in LPS-challenged RAW 264.7 cells. *J. Agric. Food Chem.,* **2009**, *57*(18), 8325-8330.
http://dx.doi.org/10.1021/jf901752f PMID: 19754168

[47] Xi, L.; Qian, Z.; Shen, X.; Wen, N.; Zhang, Y. Crocetin prevents dexamethasone-induced insulin resistance in rats. *Planta Med.,* **2005**, *71*(10), 917-922.
http://dx.doi.org/10.1055/s-2005-871248 PMID: 16254822

[48] Xi, L.; Qian, Z.; Xu, G.; Zheng, S.; Sun, S.; Wen, N.; Sheng, L.; Shi, Y.; Zhang, Y. Beneficial impact of crocetin, a carotenoid from saffron, on insulin sensitivity in fructose-fed rats. *J. Nutr. Biochem.,* **2007**, *18*(1), 64-72.
http://dx.doi.org/10.1016/j.jnutbio.2006.03.010 PMID: 16713230

[49] Nabavizadeh, F.; Salimi, E.; Sadroleslami, Z.; Karimian, S.M.; Vahedian, J. Saffron (*Crocus sativus*) increases gastric acid and pepsin secretions in rats: Role of nitric oxide (NO). *Afr. J. Pharm. Pharmacol.,* **2009**, *3*(5), 181-184.

[50] Assimopoulou, A.N.; Sinakos, Z.; Papageorgiou, V.P. Radical scavenging activity of *Crocus sativus* L. extract and its bioactive

constituents. *Phytother. Res.,* **2005**, *19*(11), 997-1000. http://dx.doi.org/10.1002/ptr.1749 PMID: 16317646

[51] Papandreou, M.A.; Kanakis, C.D.; Polissiou, M.G.; Efthimiopoulos, S.; Cordopatis, P.; Margarity, M.; Lamari, F.N. Inhibitory activity on amyloid-β aggregation and antioxidant properties of *Crocus sativus* stigmas extract and its crocin constituents. *J. Agric. Food Chem.,* **2006**, *54*(23), 8762-8768. http://dx.doi.org/10.1021/jf061932a PMID: 17090119

[52] Soeda, S.; Ochiai, T.; Shimeno, H.; Saito, H.; Abe, K.; Tanaka, H.; Shoyama, Y. Pharmacological activities of crocin in saffron. *J. Nat. Med.,* **2007**, *61*(2), 102-111. http://dx.doi.org/10.1007/s11418-006-0120-9

[53] Kanakis, C.D.; Tarantilis, P.A.; Tajmir-Riahi, H.A.; Polissiou, M.G. Crocetin, dimethylcrocetin, and safranal bind human serum albumin: stability and antioxidative properties. *J. Agric. Food Chem.,* **2007**, *55*(3), 970-977. http://dx.doi.org/10.1021/jf062638l PMID: 17263501

[54] Khazdair, M.R.; Boskabady, M.H.; Hosseini, M.; Rezaee, R.; M Tsatsakis, A. The effects of *Crocus sativus* (saffron) and its constituents on nervous system: A review. *Avicenna J. Phytomed.,* **2015**, *5*(5), 376-391. PMID: 26468457

[55] Hosseinzadeh, H.; Karimi, G.; Niapoor, M. Antidepressant effect of *Crocus sativus* L. stigma extracts and their constituents, crocin and safranal, in mice. *Proc. 1st Intl. Symposium on Saffron Biol. Biotechnol.,* **2004**, pp. 435-445. http://dx.doi.org/10.17660/ActaHortic.2004.650.54

[56] Akhondzadeh, S.; Fallah-Pour, H.; Afkham, K.; Jamshidi, A.H.; Khalighi-Cigaroudi, F. Comparison of Crocus sativus L. and imipramine in the treatment of mild to moderate depression: a pilot double-blind randomized trial [ISRCTN45683816]. *BMC Complement. Altern. Med.,* **2004**, *4*, 12. http://dx.doi.org/10.1186/1472-6882-4-12 PMID: 15341662

[57] Akhondzadeh, S.; Tahmacebi-Pour, N.; Noorbala, A.A.; Amini, H.; Fallah-Pour, H.; Jamshidi, A.H.; Khani, M. *Crocus sativus* L. in the treatment of mild to moderate depression: a double-blind, randomized and placebo-controlled trial. *Phytother. Res.,* **2005**, *19*(2), 148-151. http://dx.doi.org/10.1002/ptr.1647 PMID: 15852492

[58] Noorbala, A.A.; Akhondzadeh, S.; Tahmacebi-Pour, N.; Jamshidi, A.H. Hydro-alcoholic extract of *Crocus sativus* L. *versus* fluoxetine in the treatment of mild to moderate depression: a double-blind, randomized pilot trial. *J. Ethnopharmacol.,* **2005**, *97*(2), 281-284. http://dx.doi.org/10.1016/j.jep.2004.11.004 PMID: 15707766

[59] Moshiri, E.; Basti, A.A.; Noorbala, A.A.; Jamshidi, A.H.; Hesameddin Abbasi, S.; Akhondzadeh, S. *Crocus sativus* L. (petal) in the treatment of mild-to-moderate depression: a double-blind, randomized and placebo-controlled trial. *Phytomedicine,* **2006**, *13*(9-10), 607-611. http://dx.doi.org/10.1016/j.phymed.2006.08.006 PMID: 16979327

[60] Akhondzadeh Basti, A.; Moshiri, E.; Noorbala, A.A.; Jamshidi, A.H.; Abbasi, S.H.; Akhondzadeh, S. Comparison of petal of *Crocus sativus* L. and fluoxetine in the treatment of depressed outpatients: a pilot double-blind randomized trial. *Prog. Neuropsychopharmacol. Biol. Psychiatry,* **2007**, *31*(2), 439-442. http://dx.doi.org/10.1016/j.pnpbp.2006.11.010 PMID: 17174460

[61] Pitsikas, N.; Boultadakis, A.; Georgiadou, G.; Tarantilis, P.A.; Sakellaridis, N. Effects of the active constituents of *Crocus sativus* L., crocins, in an animal model of anxiety. *Phytomedicine,* **2008**, *15*(12), 1135-1139. http://dx.doi.org/10.1016/j.phymed.2008.06.005 PMID: 18693098

[62] Hosseinzadeh, H.; Noraei, N.B. Anxiolytic and hypnotic effect of *Crocus sativus* aqueous extract and its constituents, crocin and safranal, in mice. *Phytother. Res.,* **2009**, *23*(6), 768-774. http://dx.doi.org/10.1002/ptr.2597 PMID: 19142981

[63] 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*(1-2), 287-289. http://dx.doi.org/10.1016/S0006-8993(99)02174-5 PMID: 10642859

[64] Zhang, Y.; Shoyama, Y.; Sugiura, M.; Saito, H. Effects of *Crocus sativus* L. on the ethanol-induced impairment of passive avoidance performances in mice. *Biol. Pharm. Bull.,* **1994**, *17*(2), 217-221. http://dx.doi.org/10.1248/bpb.17.217 PMID: 8205119

[65] Tsolaki, M.; Karathanasi, E.; Lazarou, I.; Dovas, K.; Verykouki, E.; Karacostas, 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*(1), 129-133. http://dx.doi.org/10.3233/JAD-160304 PMID: 27472878

[66] 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 22-week, multicenter, randomized, double-blind controlled trial of *Crocus sativus* in the treatment of mild-to-moderate Alzheimer's disease. *Psychopharmacology (Berl.),* **2010**, *207*(4), 637-643. http://dx.doi.org/10.1007/s00213-009-1706-1 PMID: 19838862

[67] Liberati, A.; Altman, D.G.; Tetzlaff, J.; Mulrow, C.; Gøtzsche, P.C.; Ioannidis, J.P.; 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. http://dx.doi.org/10.1136/bmj.b2700 PMID: 19622552

[68] Noel-Storr, A.H.; McCleery, J.M.; Richard, E.; Ritchie, C.W.; Flicker, L.; Cullum, S.J.; Davis, D.; Quinn, T.J.; Hyde, C.; Rutjes, A.W.; Smailagic, N.; Marcus, S.; Black, S.; Blennow, K.; Brayne, C.; Fiorivanti, M.; Johnson, J.K.; Köpke, S.; Schneider, L.S.; Simmons, A.; Mattsson, N.; Zetterberg, H.; Bossuyt, P.M.; Wilcock, G.; McShane, R. Reporting standards for studies of diagnostic test accuracy in dementia: The STARDdem Initiative. *Neurology,* **2014**, *83*(4), 364-373. http://dx.doi.org/10.1212/WNL.0000000000000621 PMID: 24944261

[69] McKhann, G.M.; Knopman, D.S.; Chertkow, H.; Hyman, B.T.; Jack, C.R., Jr; Kawas, C.H.; Klunk, W.E.; Koroshetz, W.J.; Manly, J.J.; Mayeux, R.; Mohs, R.C.; Morris, J.C.; Rossor, M.N.; Scheltens, P.; Carrillo, M.C.; Thies, B.; Weintraub, S.; Phelps, C.H. The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement.,* **2011**, *7*(3), 263-269. http://dx.doi.org/10.1016/j.jalz.2011.03.005 PMID: 21514250

[70] Hannes, K.; Macaitis, K. A move to more systematic and transparent approaches in qualitative evidence synthesis: Update on a review of published papers. *Qual. Res.,* **2012**, *12*, 402-442. http://dx.doi.org/10.1177/1468794111432992

[71] Barnett-Page, E.; Thomas, J. Methods for the synthesis of qualitative research: A critical review. *BMC Med. Res. Methodol.,* **2009**, *9*, 59. http://dx.doi.org/10.1186/1471-2288-9-59 PMID: 19671152

[72] Grant, M.J.; Booth, A. A typology of reviews: An analysis of 14 review types and associated methodologies. *Health Info. Libr. J.,* **2009**, *26*(2), 91-108. http://dx.doi.org/10.1111/j.1471-1842.2009.00848.x PMID: 19490148

[73] 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*(24), 6131-6138. http://dx.doi.org/10.1021/jf300589c PMID: 22655699

[74] Lechtenberg, M.; Schepmann, D.; Niehues, M.; Hellenbrand, N.; Wünsch, B.; Hensel, A. Quality and functionality of saffron: Quality control, species assortment and affinity of extract and isolated saffron compounds to NMDA and sigma1 (sigma-1) receptors. *Planta Med.,* **2008**, *74*(7), 764-772. http://dx.doi.org/10.1055/s-2008-1074535 PMID: 18496783

[75] 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*(1), 132-138. http://dx.doi.org/10.1016/S0006-8993(97)01505-9 PMID:

9518580
[76] Sugiura, M.; Shoyama, Y.; Saito, H.; Nishiyama, N. Crocin improves the ethanol-induced impairment of learning behaviors of mice in passive avoidance tasks. *Proc. Jpn. Acad. B: Phys. Biol. Sci.,* **1995**, *71*(10), pp. 319-324. http://dx.doi.org/10.2183/pjab.71.319
[77] Abe, K.; Saito, H. Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation. *Phytother. Res.,* **2000**, *14*(3), 149-152. http://dx.doi.org/10.1002/(SICI)1099-1573(200005)14:3<149::AID-PTR665>3.0.CO;2-5 PMID: 10815004
[78] 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*, 926284. http://dx.doi.org/10.1155/2015/926284 PMID: 25713594
[79] Sekler, A.; Jiménez, J.M.; Rojo, L.; Pastene, E.; Fuentes, P.; Slachevsky, A.; Maccioni, R.B. Cognitive impairment and Alzheimer's disease: Links with oxidative stress and cholesterol metabolism. *Neuropsychiatr. Dis. Treat.,* **2008**, *4*(4), 715-722. PMID: 19043515
[80] 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*(1-2), 59-65. PMID: 20683499
[81] Naghizadeh, B.; Mansouri, M.T.; Ghorbanzadeh, B.; Farbood, Y.; Sarkaki, A. Protective effects of oral crocin against intracerebroventricular streptozotocin-induced spatial memory deficit and oxidative stress in rats. *Phytomedicine,* **2013**, *20*(6), 537-542. http://dx.doi.org/10.1016/j.phymed.2012.12.019 PMID: 23351962
[82] Walton, J.R.; Wang, M.X. APP expression, distribution and accumulation are altered by aluminum in a rodent model for Alzheimer's disease. *J. Inorg. Biochem.,* **2009**, *103*(11), 1548-1554. http://dx.doi.org/10.1016/j.jinorgbio.2009.07.027 PMID: 19818510
[83] 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. http://dx.doi.org/10.1016/j.fct.2012.11.016 PMID: 23168242
[84] 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*(2), 197-204. http://dx.doi.org/10.1016/j.bbr.2011.01.007 PMID: 21238492
[85] 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*(1-3), 222-229. http://dx.doi.org/10.1016/j.ejphar.2011.05.012 PMID: 21616066
[86] 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*(2), 956-966. PMID: 30569175
[87] Ghahghaei, A.; Bathaie, S.Z.; Bahraminejad, E. Mechanisms of the effects of crocin on aggregation and deposition of Aβ1–40 Fibrils in Alzheimer's Disease. *Int. J. Pept. Res. Ther.,* **2012**, *18*, 347-351. http://dx.doi.org/10.1007/s10989-012-9308-x
[88] 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*(3), 328-339. http://dx.doi.org/10.2478/s11658-013-0092-1 PMID: 23737042
[89] 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*(1), 79-83. http://dx.doi.org/10.1016/j.bbrc.2011.09.025 PMID: 21945434
[90] Yoshino, Y.; Ishisaka, M.; Umigai, N.; Shimazawa, M.; Tsuruma, K.; Hara, H. Crocetin prevents amyloid β1-42-induced cell death in murine hippocampal cells. *Pharmacol. Pharm.,* **2014**, *5*, 37-42. http://dx.doi.org/10.4236/pp.2014.51007
[91] 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. http://dx.doi.org/10.1016/j.cej.2016.01.035
[92] 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*(A), 47-58.
[93] Batarseh, Y.S.; Bharate, S.S.; Kumar, V.; Kumar, A.; Vishwakarma, R.A.; Bharate, S.B.; Kaddoumi, A. *Crocus sativus* extract tighten the blood-brain barrier, reduces amyloid-β load and related toxicity in 5XFAD mice. *ACS Chem. Neurosci.,* **2017**, *8*(8), 1756-1766. http://dx.doi.org/10.1021/acschemneuro.7b00101 PMID: 28471166
[94] Hadipour, M.; Kaka, G.; Bahrami, F.; Meftahi, G.H.; Pirzad Jahromi, G.; 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*(5), e22026. http://dx.doi.org/10.1002/syn.22026 PMID: 29357117
[95] 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*(5), 485-492. PMID: 26124935
[96] Rashedinia, M.; Lari, P.; Abnous, K.; Hosseinzadeh, H. Protective effect of crocin on acrolein-induced tau phosphorylation in the rat brain. *Acta Neurobiol. Exp. (Warsz.),* **2015**, *75*(2), 208-219. PMID: 26232997
[97] Akhondzadeh, S.; Sabet, M.S.; Harirchian, M.H.; Togha, M.; Cheraghmakani, H.; Razeghi, S.; Hejazi, S.Sh.; Yousefi, M.H.; Alimardani, R.; Jamshidi, A.; Zare, F.; Moradi, 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*(5), 581-588. http://dx.doi.org/10.1111/j.1365-2710.2009.01133.x PMID: 20831681
[98] Farokhnia, M.; Shafiee Sabet, M.; 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*(4), 351-359. http://dx.doi.org/10.1002/hup.2412 PMID: 25163440
[99] 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*(8), 9572-9585. http://dx.doi.org/10.1021/acsomega.8b00841 PMID: 31459089