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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 $A\beta$ 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.

Keywords: Saffron, Alzheimer's disease, treatment, neuroprotective effect, AChE inhibitor, antioxidant, anti- $A\beta$, *tau* protein inhibitor.

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 ($A\beta$) 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, dextrans, 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).

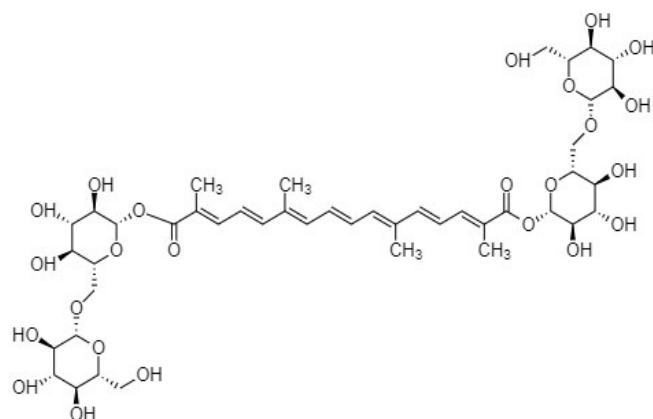


Fig. (1a). Chemical structures of crocin.

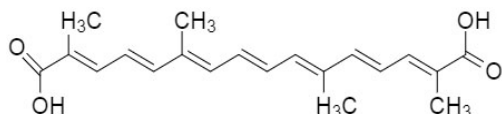


Fig. (1b). Chemical structures of crocetin.

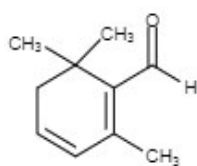


Fig. (1c). Chemical structures of safranal.

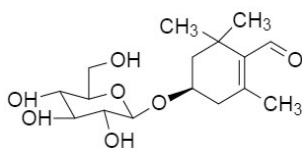


Fig. (1d). Chemical structures of picrocrocin.

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-carboxaldehyde] 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 safranin 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.

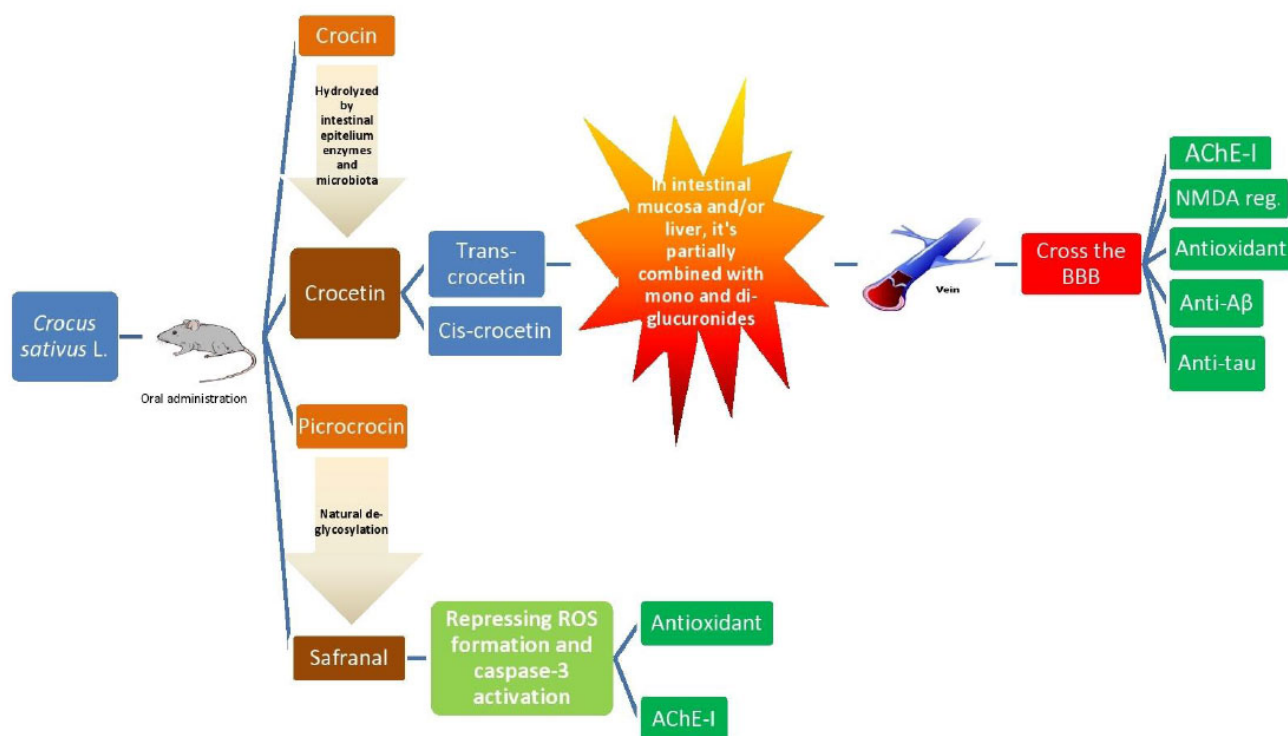


Fig. (2). Mechanisms of action of saffron extracts for the treatment of Alzheimer's disease from preclinical studies.

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.

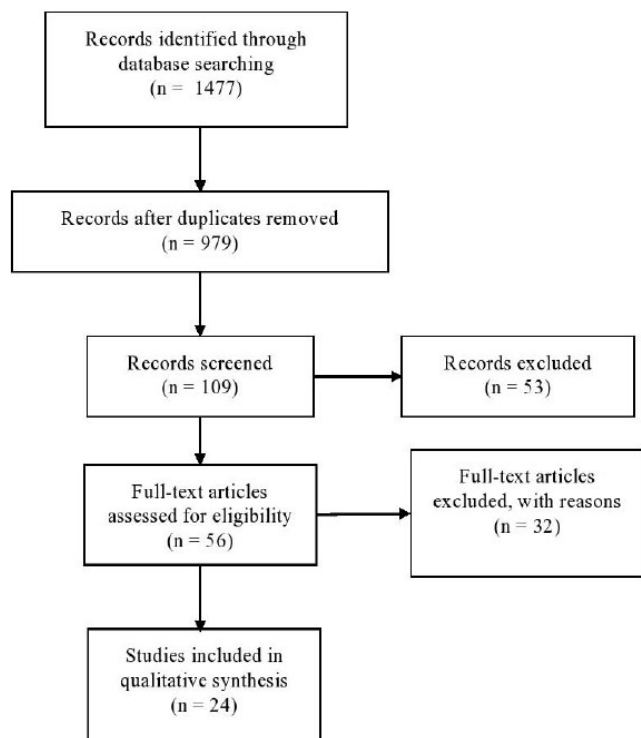


Fig. (3). Flow diagram outlining the selection procedure to identify articles which were included in the analysis of *Crocus sativus* L. (saffron) in AD treatment.

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 A β 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 AI-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 (H₂O₂), 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 (Route) or Drug Concentration | Observation | Species | Pharmacological Effects | References |
|--------------------|------------------------------------------|--------------------------------------------------------------|-----------------|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| AChE-I | Safranal Crocetin Dimethylcrocetin | 96.33 µM 107.1 µM 21.09 µM | <i>In vitro</i> | - | 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 | 1–10 µM | <i>In vitro</i> | - | Saffron extracts and crocetin had a high affinity to the phenylcyclidine binding site of the NMDA receptor and at the sigma(1) receptor, while the crocins and picrocrocins were not effective | [74] |
| | Crocetin Crocetin Picrocrocetin | 1–10 µM 1–10 µM 1–10 µM | | | | |
| | Crocetin Picrocrocetin | 50–100–200mg/kg (p.o. acute) 50–100–200mg/kg (p.o. acute) | <i>In vivo</i> | Mouse | Only crocin reversed ethanol-induced deficits | [75] |
| | Crocetin | 50–200 mg/kg (p.o. acute) | <i>In vivo</i> | Rat | Treatment improved the memory impairment in ethanol-treated rats in dose-dependent method. | [76] |
| Antioxidant | Crocetin | 15–30mg/kg (i.p. chronic) | <i>In vivo</i> | Rat | Treatment of 30mg/kg for three weeks reversed streptozotocin-induced performance deficits in the Y-maze and passive avoidance task | [80] |
| | Crocetin | 100mg/kg (p.o. chronic) | <i>In vivo</i> | Rat | Treatment with crocetin for 21 days reversed streptozotocin-induced spatial memory deficits | [81] |
| | Crocus sativus extract | 60mg/kg (i.p. chronic) | <i>In vivo</i> | Mouse | Ineffective to reverse AlCl ₃ -induced memory deficits | [83] |
| | Crocus sativus extract | 60mg/kg (i.p. chronic) | <i>In vivo</i> | Aged mouse | Treatment for 7 days reversed age-related memory deficits | [84] |
| | Crocus sativus extract Crocetin | 30mg/kg (i.p. chronic) 15–30mg/kg (i.p. chronic) | <i>In vivo</i> | Rat | Treatment for 21 days reversed chronic stress-induced | [85] |
| | Crocetin | 5 or 20 mg/kg (i.p. chronic) | <i>In vivo</i> | 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 | <i>In vitro</i> | - | Trans-crocetin-4 inhibited Aβ fibrillogenesis at lower concentrations than dimethylcrocetin | [51] |
| | Crocetin | 15 µg/mL | <i>In vitro</i> | - | Crocetin decreased the number of fibrils formed and significantly reduced the average fibril length of Aβ ₄₀ | [87] |
| | Crocetin | 15.4 µM | <i>In vitro</i> | - | Crocetin allowed the inhibition of Aβ ₄₂ -mediated amyloid fibril formation, and also the disruption of amyloid aggregates | [88] |
| | Crocetin | 174 µM | <i>In vitro</i> | - | Crocetin inhibited Aβ fibril formation, destabilized pre-formed Aβ fibrils, stabilized Aβ oligomers and prevented their conversion into Aβ fibrils | [89] |
| | Crocetin | 1 – 10 µM | <i>In vitro</i> | - | Crocetin at 1 – 10 µM protected HT22 cells against Aβ _{1–42} -induced neuronal cell death and decreased ROS production increased by Aβ _{1–42} | [90] |

(Table 1) contd....

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

Abbreviations: **ACHE-I**, acetylcholinesterase inhibitors; **NMDA**, N-methyl-D-aspartate receptor; **AlCl₃**, aluminium; **$A\beta$** , 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 $A\beta$ 1-42 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 $A\beta$ 1-42 deposition in the hippocampus of the brains of treated mice compared with untreated mice.

3.1.3. Effects on $A\beta$ Peptide and tau Protein

Several *in vitro* studies have been performed on the effect of saffron extract and crocin on $A\beta$ 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 crocin, able to pass through BBB, inhibits fibril $A\beta$ formation, destabilizes pre-formed $A\beta$ fibrils, causes $A\beta$ oligomers stabilization [89].

Another study has demonstrated that crocin owns a strong neuroprotective effect vs. $A\beta$ ₁₋₄₂-induced cytotoxicity in hippocampal cells through oxidative stress reducing [90]. It was shown that crocin at 1-10 μ M protected HT22 cells vs. $A\beta$ ₁₋₄₂-induced neuronal cell death and decreased ROS

production. In an *in vitro* study, a neuronal membrane bioreactor was used as a model of $A\beta$ -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 $A\beta$ peptide and crocin induced a significant dose-dependent inhibition of ROS production and apoptosis, highlighting the crocin skill to avoid the $A\beta$ 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 $A\beta$ 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 $A\beta$ and a significant reduction in $A\beta$ ₄₀, the most toxic and pathogenic form of $A\beta$. Furthermore, in brain homogenates, crocin significantly improved $A\beta$ expression declining neprilysin (NEP) enzyme and adenosine triphosphate binding cassette subfamily A member 1 (ABCA1), a regulatory protein involved in $A\beta$ clearance. These results show that crocin could employ its protective effect against $A\beta$ pathology, through the reduction of the brain load of $A\beta$, including toxic oligomers and an increased expression of the degrading enzymes and ABCA1. The effects of crocin on $A\beta$ -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 $A\beta$ 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 $A\beta$ toxicity.

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

An *in vitro* study has reported the crocin inhibitory effect on aggregation of recombinant human τ 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 (Route) | Severity of Disease | Pharmacological Effects | References |
|------------------------|----------------|----------------------------------------------------|----------------------------|---------------------------|-----------------------------------------------------------------------|------------|
| 12-month, Single-blind | 35 | <i>Crocus sativus</i> 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) Crocetin (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) Crocetin (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 | Crocetin (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, Aβ 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: $F = 4.12$, d.f. = 1, $p = 0.04$), 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 administered 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 IIM-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 IIM-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

Not applicable.

FUNDING

This work was fully supported by “Ministero della Salute”, I.R.C.C.S. Research Program, Ricerca Corrente 2018-2020, Linea n. 2 “Malattie complesse e terapie innovative” and by the “5 x 1000” voluntary contribution.

CONFLICT OF INTEREST

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

ACKNOWLEDGEMENTS

All authors contributed to the analysis, drafting the paper, and the conclusions.

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