

Review Article

Saffron the “Red Gold” and Its CNS Activity: A Challenge for Future Applications in Nutraceuticals

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Saffron, indicated as “the red gold,” is an extraordinary spice with a unique aroma and organoleptic properties that is largely diffused in food preparation as well as a traditional medicine in many countries of the world. In the last years, several studies have considered this spice for CNS-based disorders showing its potential usefulness. In this review, we considered the role of saffron as a nutraceutical for the management of the most diffused CNS diseases considering the important role of oxidative stress on the pathogenesis of such diseases. In fact, recent findings support a crucial role of oxidative stress in different CNS diseases suggesting an important role of antioxidants. Preclinical and clinical evidence of its efficacy in different physiopathological pathways involved in several CNS diseases were discussed showing evidence of pharmacological activities and beneficial effects in pathological models or in small trials. Due to low toxicity and significant activities on oxidative stress and inflammation as well as the ability to modulate mitochondrial function, the saffron extracts and their constituents appear to be promising nutraceutical active compounds in this area. Further investigations are in progress to assess the efficacy and safety of preventive agents as nutraceuticals or as adjuvant compounds to be used in combinations with other therapeutic approaches. Saffron nutraceuticals with significant antioxidant activity can be useful in improving the quality of life of patients suffering from several different pathological conditions related to CNS. In this review, we summarized the more recent studies showing that standardized saffron products can be a valuable instrument of well-being due to their effects on multiple targets that support the health of the brain and related tissues.

1. Introduction

Crocus sativus L. is a perennial stemless herb of the Iridaceae family that is cultivated in various parts of the world. In Europe, France, Italy, Spain, Greece, and Turkey are known for saffron production. Other very important producers are Iran, Azerbaijan, and very big countries such as India and China. The plant reaches approximately 10–25 cm in height and it is, in general, developed from a corm (bulbous tuberous structure) replicated every year. The flower consists

of six tepals, three stamens, and a style, which culminates with three red-branched stigmas. The spice that is indicated as “saffron” is obtained from the dried stigma of *Crocus sativus* L. and is one of the most highly valued agricultural products that is used for cooking purposes to give flavor, color, and aroma to food [1–5]. Saffron is known as “red gold” because of its high price; its value can be \$40–80 per gram. This cost is mainly caused by manual operations for plucking saffron flowers during harvesting, requesting manual work [6]. Further reasons for the high cost are the

time-consuming cultivation and the need for a large number of human resources. Also, the high amount of plant material needed can be approximately calculated as 80 kg of *C. sativus* flowers; nearly 500,000 stigmas are required to produce 1 kg of dried saffron [6]. The cultivation of *Crocus* species for culinary and medicinal utilization has been performed since ancient times, and a long tradition is present in Greece and Iran. Due to commercial exchanges, the use of this spice was spread worldwide being diffused into the Mediterranean region, Eastern Europe, South Asia, and China [2]. There are many traditional applications of *C. sativus* as it is used in food and cosmetics as well as in several traditional medicine preparations. In Persian traditional medicine, saffron was used as a tonic, and many preparations have indications, especially for protecting the vascular and nervous systems. Saffron is a famous spice and a remedy largely described in the traditional medicine of several countries. Many medicinal uses are reported in textbooks in the traditional receipts of herbal medicine. The main area of use is to treat spasms, menstrual and other pains, and digestive ailments to improve liver function [7]. Saffron is a valuable ingredient in different remedies for the treatment of depression, insomnia, measles, and dysentery. [2]. Furthermore, this species has been indicated for insomnia as well as a stimulant, aphrodisiac, antidepressant, and supportive treatment of cancer, thus suggesting that it can have several effects on the central nervous system (CNS).

In folk medicine, saffron is claimed not only for different applications ranging from euphoric to carminative to stomachic but also as an antispasmodic, antidiarrheal, and expectorant. Other reported uses suggest its application as a sedative, diaphoretic, stimulant, and aphrodisiac showing large diffusion in many countries [1, 2, 6]. The extract obtained from saffron and its active phytoconstituents have been considered for their potential usefulness as anticonvulsants, antidepressants, and anti-inflammatory agents [5]. Saffron's claimed effects can be related to its complex group of constituents ranging from the aldehydic compound safranal to the carotenoids related to crocetin and crocins as the most known. Some preclinical studies have evidenced some possible antidepressant effects of crocin and crocetin, two of the major pigments of saffron [5, 8, 9]. Clinical pilot studies evidenced the antidepressant effects of saffron extracts, but standardization and chemical composition can be a challenge. Furthermore, more extensive studies are needed to establish possible efficacy in this area [8].

In recent years, the role of oxidative stress (OS) has been highly considered especially related to CNS diseases and mostly to neurodegenerative diseases (NDDs).

In NDDs, as in many other human diseases, the OS can be defined as an imbalance between the ROS production and antioxidant capacity of the cell. One of the essential ATP sources is oxidative phosphorylation, which occurs in mitochondria and generates free radicals, reactive nitrogen species (RNS), and carbon- and sulfur-centered radicals. OS induces damages in many of the important biomolecules such as nucleic acid and protein. Furthermore, OS induces lipid oxidation and contributes to the formation of advanced glycation end products. The strong role of OS is also

recognised in glial cell activation, apoptosis, and proteasome dysfunction and can be one of the causes of mitochondrial dysfunction. Other mechanisms related to the effect of OS on CNS tissue can be the appearance of defects in the ubiquitin-proteasome system and the induction of oligomerization of alpha-synuclein (α Syn) or beta-amyloid ($A\beta$) involved in chronic CNS diseases. The OS can cause induction of cytokine and increase of the inflammatory response and can be related to the damage of the blood-brain barrier. All these mechanisms can strongly afflict the function of CNS also considering the peculiarity of this tissue that makes it particularly vulnerable to oxidative damage. In fact, the brain is characterized by high energy consumption and metabolic demand, and small imbalances can generate tissue damage and induce neuroinflammation. CNS metabolisms consume nearly 20% of the oxygen of the body in normal conditions, and for this reason, there is a large mitochondrial activity with physiologically significant production of radical species of oxygen (ROS). A further point is that the brain and CNS tissue have a high content of lipids, which can obviously be subjected to the peroxidation induced by ROS, and metals. Finally, the low antioxidant defense of neurons and their reduced regenerative capacity should be considered to fully understand the high susceptibility of this tissue to OS damage [10].

The structural and functional perturbations in the brain that are associated with depression structure can be related to OS. Preclinical and clinical studies have shown that increased ROS generation and consumption of antioxidative defenses are key points in the altered brain structure, and this is considered the basis for the "oxidative stress hypothesis of depressive disorders," and the excess of OS has emerged as a major cause of the depressive disorder [11]. In this regard, a significant role in the management of depression can be suggested for all those compounds as herbs and natural products known to possess a substantial antioxidant capacity. Due to its peculiar chemical composition, saffron and several of its antioxidant constituents may result as promising agents useful for the management of CNS disease and, for example, as an antidepressant. Furthermore, thanks to their antioxidant activity as well as their possible action on mitochondria, saffron constituents can be considered significant in further studies. Finally, due to their importance in many CNS diseases of oxidative stress and management of cellular function, particular emphasis will be dedicated to potential targets of saffron constituents on mitochondria.

Due to its extensive use as a spice and the recent advances in research, saffron can be considered a valuable natural product useful for developing nutraceuticals and/or remedies focused on the CNS. In this review, we will summarise the most recent findings related to saffron and its possible uses for CNS considering the improvement in product obtaining, standardization, and trials related to bioavailability as well as any clinical or pilot study showing potential usefulness.

In a recent review, nutritional and health-beneficial properties of saffron (*Crocus sativus* L.) were taken into account showing the literature data related to its marker compounds, the bioavailability and bioaccessibility of the

putative active compounds, and the bioactivity of saffron phytochemicals summarising some of the therapeutic effect of this spice useful to counteract different ailments [6]. The authors considered all the parts of the botanical species focusing also on the possible uses and exploitation of other flower parts. In this review, we will focus mainly on the literature published in the last five years (2017–2023). In the first part of the manuscript, the most studied bioactivities of saffron in CNS diseases are reported, and in the second part, clinical applications of saffron are discussed. We also discuss the potential usefulness of saffron for CNS-related ailments, focusing on a perspective of nutritional supplements and nutraceutical intervention.

2. Methods

The literature search was performed using Scopus, PubMed, ScienceDirect, ACS publication, and MEDLINE databases, as well as Wiley Online Library for published articles in the English language, mostly referring to the period from 2015 to 2023. The search terms included *Crocus sativus*, crocus constituents, CNS system disease, antioxidant, depression, nutraceutical, and food supplements. More relevant papers were selected, and relevant reviews of the last 4 years were considered to prepare manuscripts focusing on different aims compared to the previously published reviews.

3. Main Text

The efficacy in the prevention and protection of CNS of crocus preparation is strictly related to the chemical constituents that can be determined in the plant materials. Crocus stigmas are rich in different classes of secondary metabolites that are responsible for the color and the organoleptic properties of this spice [6, 12, 13]. There are several papers considering the saffron composition, and literature indicates that more than 150 volatile and non-volatile compounds have been reported belonging to several chemical classes as proteins, amino acids, carbohydrates, minerals, vitamins, and pigments [14]. A review summarising the contents of bioactive constituents of the plants of the species crocus was recently published [15]. Details on chemical constituents of saffron are well described in other papers [4, 16] and are summarized in the supplementary materials included in the present review in which we report structures, classes of compounds, and their contents.

3.1. Bioactivity of Saffron Related to CNS Diseases. Several studies have considered the potential application of saffron and its constituents as a treatment for CNS-related diseases. A few different experimental models and approaches have been proposed, but in the last years, research focused mostly on Alzheimer's disease and Parkinson's disease. Several authors have recently published reviews on the topic focusing on the saffron or its constituents [17, 18]. Recent paper reported potential mechanisms related to the possible effects of saffron or saffron constituents on some CNS diseases and the most relevant ones are summarized in Table 1.

The summarized paper reported in Table 1 presents some examples of the significant effects of saffron in the field of the most significant CNS diseases considering both neurodegenerative pathologies and depression and anxiety.

Furthermore, several papers considered the possibility of clinical applications, and some of the general uses are summarized in Table 2, where the findings related to some of the relevant CNS diseases are reported.

Thus, the literature on saffron revealed that there is a relatively large amount of published literature related to its possible use in CNS-related diseases and most of the proposed references indicate the efficacy and safety of the used remedies (Figure 1).

C. sativus L. possesses several pharmacological properties mainly attributed to its biologically active substances which are part of the class of terpenoids and include crocin, safranal, and picrocrocin. Increasing scientific evidence considers *C. sativus* L. as a medicinal plant with antioxidant, antiatherosclerotic, anti-inflammatory, hypotensive, hypolipidemic, antidiabetic, antidepressant, anxiolytic, and hypnotic characteristics [5, 9, 13, 32]. As demonstrated in several animal studies, the constituents of *C. sativus* L. are relatively safe considering the data available on acute, subacute, subchronic, and chronic exposure. The data also indicate that saffron and its metabolites present limited toxicity [1, 2, 6]. The major constituents of saffron, safranal, crocetin, and crocin in animal models with various disorders associated with oxidative stress system, immune system imbalance, and inflammation have demonstrated antioxidant, immunomodulatory, and anti-inflammatory effects, respectively. The effects of this plant have also been indicated by *in vitro* models, which confirmed the anti-inflammatory, immunomodulatory, and antioxidant effects [2, 6, 13, 33, 34]. Aluminum (Al) exposure is one of the environmental risk factors possibly related to the pathogenesis of neurodegenerative disease. Saffron extract was studied as a protective agent against aluminum maltolate (Almal)-induced oxidative stress and apoptosis in PC12 cells. The results showed a dose-dependent activity [35].

Considering the CNS-directed effects, recent studies have suggested the correlation between oxidative stress and depression [11]. In particular, some ideas underpinning this connection should be highlighted. First, due to its high oxygen consumption and higher lipid content, the brain is vulnerable to OS. Second, OS is a cause of neurodegeneration, and its involvement in the pathogenesis of a major depressive disorder is well established. OS and proinflammatory signaling have emerged as mainstays in the pathogenesis of MDD (major depressive disorder). Thus, targeting these aberrant alterations with suitable antioxidants could be an effective strategy to manage MDD [11]. In this regard, the studies supporting the antioxidant effects of saffron can be taken into account for CNS pathologies. As reported in a comprehensive review [14], the effects of the main constituents of *C. sativus* on oxidative, inflammatory, and immune responses in neurodegenerative, respiratory, coronary, gastrointestinal, hematological, and urinary system diseases have been studied, also related to the biochemical pathways involved. The potent antioxidant activity of *C. sativus* stigma is mainly due to the presence of safranal, crocin, and crocetin. *C. sativus* and its constituents eliminate

TABLE 1: A summary of the most recent studies considering specific effects of crocus on CNS diseases.

| Disease and organism | Compound or extract and main findings | Reference |
|--|--|-----------|
| Male Wistar rats, microinjection of beta-amyloid, <i>in vivo</i> model of AD | Safranal 0.025, 0.1, and 0.2 ml/kg prevented learning and memory decline via neuronal protection | [19] |
| 5XFAD mice used as an AD model | <i>C. sativus</i> extract (50 mg/kg/day, added to mice diet). Positive effect of <i>C. sativus</i> against AD by reducing A β pathological manifestations | [20] |
| Rat with microinjection of beta-amyloid, <i>in vivo</i> A β models of the AD | Crocetin improved amyloid beta-induced long-term potentiation and memory deficits in the hippocampal CA1 neurons in freely moving rats | [21] |
| Mice with induced PD | Saffron was administered before inducing Parkinson. RNA microarray analysis of the brain transcriptome revealed differential expression of 424 genes. Bioinformatics analysis identified the enrichment of molecular pathways and expression changes in candidate genes with known neuroprotective actions | [22] |
| Rat model of depression and anxiety | Crocetin and crocetin induced changes in serotonin and corticosterone serum concentrations and decreased hippocampal NR2B expression. Furthermore, crocetin inhibited the NMDA receptor. Behavioral tests for depression (sucrose preference test and forced swimming test) and anxiety (open field test) | [23] |

AD, Alzheimer's disease; PD, Parkinson's disease.

TABLE 2: Main paper and findings considering possible clinical applications of saffron on CNS diseases.

| Topic | Findings | Reference |
|---|--|-----------|
| Preclinical and clinical data on AD review | 1477 studies published until November 2020 were considered and 24 met the inclusion criteria for the review. Only 4 studies 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. So further studies are needed to establish the possible role of saffron in AD | [24] |
| Mild to moderate depression clinical | Saffron was effective for treating mild to moderate depression and had comparable efficacy to synthetic antidepressants | [25] |
| Multiple sclerosis review | Saffron may prove beneficial in improving antioxidant defense and oxidative stress in patients with MS; however, the evidence appears scattered, heterogeneous, and inadequate, so trials of better design and MS-specific outcomes are required | [26] |
| Adults with poor sleep, sleep quality, cortisol, and melatonin concentrations | Supplementation with standardized extract (Affron®) 14 mg or 28 mg. The authors reported sleep-enhancing effects of 28 days with saffron supplementation in adults | [27] |
| <i>C. sativus</i> for insomnia | Although there is limited evidence of a very low to moderate quality, <i>C. sativus</i> may benefit people with insomnia | [28] |
| Adjunctive therapy in adults with attention-deficit/hyperactivity disorder | Saffron combination therapy with Ritalin can effectively improve symptoms of patients with ADHD | [29] |
| Management of premenstrual dysphoric disorder | 15 mg of saffron for 2 weeks in the luteal phase of two menstruation cycles. Treatment was an efficacious herbal agent for the treatment of PMDD with minimal adverse effects | [30] |
| Insomnia | Intranasal formulation containing oily macerates of <i>Viola odorata</i> L., <i>Crocus sativus</i> L., and <i>Lactuca sativa</i> L. 0.02 mg/mL crocin and 4 µg/mL isoquercitrin | [31] |

AD, Alzheimer's disease; PD, Parkinson's disease.

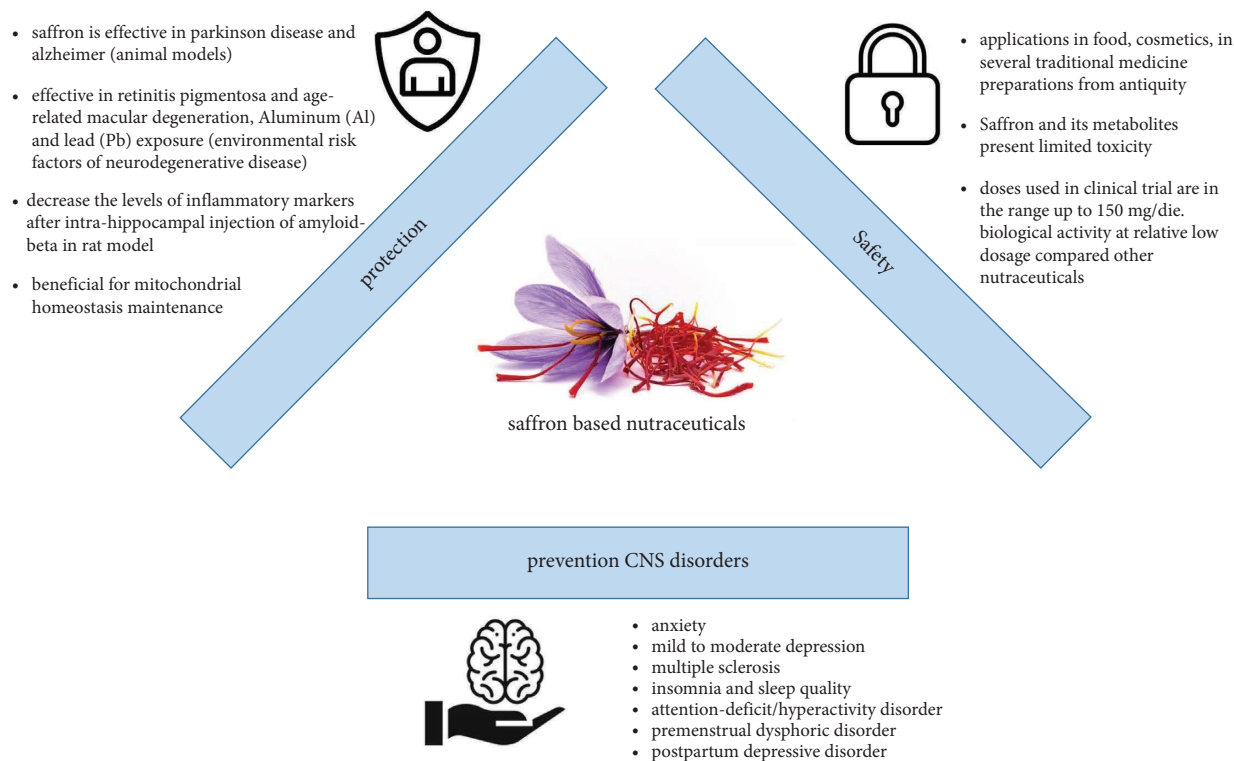


FIGURE 1: Saffron as a possible nutraceutical for CNS-related diseases due to its efficacy and safety profile.

ROS, stabilize cell membranes, and decrease the peroxidation of unsaturated membrane lipids [14]. The antioxidant properties of saffron constituents have been extensively studied and considered in many reviews [7, 13, 32, 33, 36–38], and all data indicate that crocins and flavonoids are very well established as antioxidants and their importance in different biochemical pathways involved in the antioxidant response of organism has been evidenced. An intranasal formulation containing violet oil, saffron oil, and lettuce seed oil was studied for the effects on insomnia on fifty patients with primary chronic insomnia. The authors concluded by affirming that the intranasal use of the multitherbal preparation can be used to improve chronic insomnia and to reduce the dose of conventional hypnotic medications in insomniac patients [31].

3.2. Mechanism of Actions Supporting Saffron Activities on CNS. Several papers reported specific mechanisms of action of saffron extracts and their constituents. Due to the peculiarity of the nervous tissue, the specific cellular model was developed to recreate *in vitro* the most similar pathophysiological condition of specific neuron groups. The neuroprotective mechanism of saffron was related to its direct action on the P2X7 ionotropic receptor (P2X7R), and its alteration is related to retinal neurodegenerative disorders such as retinitis pigmentosa and age-related macular degeneration. Saffron protects photoreceptors from ATP-induced cytotoxicity. Two fractions isolated from saffron stigmas, picrocrocin/kaempferol and crocin, were used for parallel experiments on 661W (immortalized cone photoreceptor cell line derived from the retinal tumor of a mouse)

and human embryonic kidney 293 cells expressing P2X7R. The first cellular model was derived from retinal tumors of a transgenic mouse line and showed biochemical properties and cellular properties of photoreceptors, while the P2X7 receptor was proposed as a potential therapeutic target in central nervous system (CNS) diseases. High levels of extracellular ATP in the retina could be the cause of retinal neurodegeneration and saffron demonstrated a direct action on this receptor and, in both cellular models, the presence of crocin guaranteed a high level of protection from stress induced by the ATP extension [39].

Another cellular model was developed to study the fibrillation propensity of α -synuclein, a presynaptic neuronal protein, that can trigger Parkinson's disease (PD). Specifically, the E46K mutation of the α -synuclein gene has been linked to autosomal dominant early-onset PD. Crocin was studied to evaluate its ability to reduce the formation of the amyloid fibril of α -synuclein E46K. Crocin dose dependently inhibited the amyloid formation by incubating α -synuclein E46K as demonstrated by the reduction of the fluorescence intensity of thioflavin-T. Static light scattering also demonstrated that crocin caused a significant reduction in the aggregation of E46K α -synuclein [40].

PC12 cells are a type of catecholaminergic cells that synthesize, store, and release norepinephrine and dopamine and are used as a model to study apoptosis in neuronal cells. PC12 cells were examined to evaluate the effects of crocin on serum/glucose-deprived cultures versus α -tocopherol used as a reference antioxidant. The culture of glucose-deprived PC12 cells had a significant change in morphology and caused peroxidation of membrane lipids and decreased

intracellular superoxide dismutase (SOD) activity. The oxidative stress induced the transfer of phosphatidylserine (PS) residues across the outer membrane, and these can be used as an early marker of apoptotic induction. Treatment with crocin kept the cell morphology more intact, even compared to the action of α -tocopherol. Crocin significantly reduced membrane-level lipid peroxidation and restored SOD activity. Crocin, therefore, appears to have a fundamental role in the modulation of antioxidant effects thanks to its SOD-restoring activity. Crocin also suppressed caspase-8 activation caused by serum/glucose deprivation in a concentration-dependent manner (0.1–10 μ M). Crocin did not inhibit caspase-8 activity in cell lysates and its inhibitory effect may be indirectly caused by its antioxidant activity [41]. Thus, the antioxidant effect of crocin is not only a direct radical scavenger but also can help SOD restoration.

The effects of saffron components on the activity of N-methyl-D-aspartate (NMDA), which plays an important role in regulating glutamate levels in the brain, have been analyzed. Some of these findings were summarized in a recent review focused on saffron and Alzheimer's. Crocin antagonizes the inhibitory effect of ethanol on NMDA receptor-mediated responses in the dentate gyrus of rat hippocampal slices [24].

The effects of *C. sativus* extract and its main active ingredients were evaluated *in vitro* on the blood-brain barrier (BBB) function and integrity and on $A\beta$ -related pathology. In addition, the extract was studied in 5XFAD, a mouse model for AD to evaluate its possible usefulness. *In vitro* results showed that the *C. sativus* extract increased the maintenance of membrane stability of a cell-based BBB model and improved $A\beta$ transport. Saffron inhibited tau aggregation with an IC_{50} of 100 μ g/mL [20].

3.2.1. Inflammation. Crocins can be considered potent anti-inflammatory compounds, and many works reported their activity in this regard. Some of the most important mechanisms are related to the inhibition of cyclooxygenase-1 (COX1) and cyclooxygenase-2 (COX2) and the blocking of prostaglandin-2 (PGE2) production by inhibiting the nuclear translocation of NF- κ B p50 and p65 subunits [5]. The bioactive compounds crocin, crocetin, and safranal show encouraging effects in the prevention of diseases related to oxidative stress, such as cardiovascular and respiratory diseases, cancer, and neurological disorders [12].

Neurodegenerative diseases are characterized by the presence of inflammatory markers including IL-1 β , IL-6, IL-8, TNF- α , and NF- κ B [42]. A study of cognitive deficits after an intrahippocampal injection of amyloid-beta ($A\beta$ 1-40) in rats showed the beneficial effects of safranal treatment by decreasing the levels of inflammatory markers [19]. Hippocampal $A\beta$ 1-40 lesions increase the levels of deleterious free radicals and mitochondrial dysfunction. After safranal treatment, mitochondria reestablish the membrane potential suggesting a beneficial effect of saffron against mitochondrial dysfunction [19]. The activity of crocins was studied in HT22 cells and Alzheimer's disease mouse model. In particular, positive effects were observed in l-Glu-damaged

HT22 cells; moreover, the ability of crocin to improve memory abilities and cognitive functions was verified in AlCl₃ and d-gal-induced AD mice [43].

The protective effect of safranal on toxicity and oxidative damage induced by beta-amyloid ($A\beta$) and hydrogen peroxide was studied in PC12 cells as a model of Alzheimer's cell damage. When PC12 cells are exposed to amyloid-beta, safranal treatment reduces amyloid-beta-induced apoptosis via the P13K/AKT and MAPK/ERK pathways [43], thus, demonstrating safranal protection against free radicals produced by H₂O₂. [43]. Studies revealed that lead exposure could be a risk factor in the development of Parkinson's disease, and for this reason, the possible therapeutic potential of *C. sativus* was evaluated. In detail, *C. sativus* (50 mg/kg BW) was administered by oral gavage to counteract the neurotoxic effect of Pb (25 mg/kg BW i.p) for three consecutive days on the dopaminergic system and locomotor performance in *Meriones shawi*. Animals treated with saffron presented protection, and their locomotor activity was restored significantly compared to the control group [43].

Chronic restrictions can induce depressive behaviors in mice. The effects of saffron on depression as well as its neuroprotective and pharmacological effects on the intestinal function of crocetin in mice exposed to chronic restraint stress were studied [43]. Administration of a crocetin-containing extract attenuated the levels of MKP-1, proBDNF, alanine transaminase, and aspartate transaminase and increased serum dopamine and CREB levels. Histopathological analysis showed that crocetin attenuated hippocampal damage in stressed mice and demonstrated a protective effect in neuronal cells. Immunofluorescent and WB analyses showed increased expression levels of ERK1/2 and CREB and inhibited expression levels of MKP-1 and proBDNF in the hippocampus. After treatment with crocetin, the intestinal ecosystem partially recovered to the level of the control group. This work concludes that crocetin has potential neuroprotective properties and ameliorates the effects of stress-related brain damage by regulating MKP-1-ERK1/2-CREB signaling and the intestinal ecosystem [43].

Several *in vitro* studies and many *in vivo* studies describe saffron as an important antioxidant agent and its efficacy seems to be attributed to the synergy of its bioactive ingredients.

3.2.2. Mitochondria and Saffron. Mitochondria play different key roles in the cell. Among their multiple duties, their primary function is ATP production through oxidative phosphorylation. Even though mitochondrial density in neurons is not the highest, the brain consumes ten times more glucose and oxygen than other tissues; thus, it is not surprising that mitochondrial dysfunctions are linked with several neurological pathologies. Furthermore, it is worth mentioning that, on the one hand, mitochondria play a role in the catabolism of carbon-rich fuel molecules; on the other hand, they control the intrinsic apoptotic pathway. Thus, mitochondria act as yin and yang, simultaneously coordinating cell growth and cell death pathways [44].

Mitochondria are the primary source of reactive oxygen species (ROS) formation in the form of hydrogen peroxide and superoxide anion; indeed, the function of the mitochondrial

transport chain is coupled with ROS production [45]. In terms of mitochondrial ROS overproduction, the increased oxidative stress could trigger mitochondrial damage resulting in the initiation of apoptosis in cells, especially in nonmitotic and long-living cells, such as neurons. In addition, enhanced oxidative stress and decreased antioxidant capacity are linked with several CNS disorders, such as neurodegenerative diseases; thus, the crucial role of endogenous or exogenous antioxidant molecules is not up for discussion.

Herein, we will discuss the role of saffron in ameliorating mitochondrial activity by decreasing neuronal cell death and enhancing antioxidant defenses in different pathological conditions.

In the last two decades, several works focused on Parkinson's disease (PD), a well-established neurodegenerative condition of the CNS characterized by uncontrollable movements and balance and coordination difficulties, which are caused by extensive cell death of neurons located in the substantia nigra pars compacta. It has been demonstrated that crocetin treatment enhances the antioxidant capacity decreasing neuronal cell death in the substantia nigra of a rat model for PD [46]. Notably, the brain transcriptome of a PD mouse model treated with saffron extract shows increased expression of genes linked with neuroprotective activity; several mitochondrial genes linked with antioxidant defense were found [22]. Moving to an easier *in vivo* model, such as *Drosophila melanogaster*, rotenone-induced neurotoxicity, which specifically triggers mitochondrial dysfunction, is reduced in a fly model of PD by saffron methanolic extract treatment. Interestingly, saffron treatment rescues locomotor activity in rotenone-treated flies [47]. Successively, similar experiments were carried out in a mouse model of neurotoxin-induced PD; also in this case, daily crocin treatment is sufficient to attenuate the rotenone-induced neurotoxic effect in the striatum. In particular, crocin-treated mice show decreased anxiety and increased motor coordination and exploratory behavior. Mainly, enhanced levels of mitochondrial enzymes were detected in the striatum, supporting saffron's positive effect on mitochondrial functionality [48]. The ability of saffron to sustain mitochondrial activity was also confirmed in a study on depression. In detail, crocin-I preserves oxidative phosphorylation and ATP production by attenuating oxidative markers in the hippocampus of the chronic corticosterone-induced depression mouse model [49].

Finally, a few studies underlined the crucial role of saffron in apoptosis regulation. It has been demonstrated that crocin exhibits antiapoptotic behavior modulating the expression of Bcl-2 family proteins in neuronally differentiated PC12 cells [50]. Crocetin can also counteract photoreceptor degeneration and retinal dysfunction through caspase-3 and caspase-9 inhibition [51]. Importantly, this peculiar antiapoptotic activity of saffron was also demonstrated in two different *in vivo* models of Alzheimer's disease, another common neurodegenerative disorder; specifically, hippocampal and intraperitoneal crocin administration is sufficient to inhibit beta-amyloid-induced apoptosis [21, 52].

These data indicate that mitochondrial activity is involved in the protective effect of saffron on cell death and oxidative damage in the CNS (Figure 2). However, even if saffron is used in Chinese traditional medicine and it well established its antioxidant capacity, little is known about the mitochondrial molecular mechanisms involved in this scenario. Thus, more effort should be made to unveil specific mitochondrial pathways regulated by saffron.

3.3. Clinical Applications of Saffron (*Crocus sativus* L.) and Its Constituents in CNS Disorders. Saffron and its active biomolecules such as crocin, crocetin, and safranal have shown several clinical applications in CNS disorders. Saffron extract has been studied in more than twenty antidepressant clinical trials versus placebo or standard therapy, showing effectiveness as an antidepressant drug. An international task force of The World Federation of Societies of Biological Psychiatry (WFSBP) and the Canadian Network for Mood and Anxiety Disorders (CANMAT), involving 31 clinicians and leading academics from 15 countries, indicated saffron extract as a complementary treatment in depression [53].

Besides antidepressive activity, many clinical trials investigating saffron's hypnotic, antianxiety, and anti-Alzheimer properties also demonstrated effectiveness compared to placebo or other treatments [24, 25, 54]. Moreover, novel promising therapeutic actions of saffron are becoming available in relevant CNS disorders such as multiple sclerosis (MS), attention-deficit/hyperactivity disorder (ADHD), and mild cognitive impairment (MCI) [26, 55, 56].

3.3.1. Depression and Anxiety. Depression and anxiety are two of the prevalent mood disorders and the most relevant causes of disability worldwide [57]. Although several drugs have been approved for these conditions, their use is often associated with severe side effects or inefficacy. In addition to medical treatment, in recent years, plant-derived products are providing interesting applications both alone in the early phase of the diseases and in association with psychiatric drugs through a network pharmacology approach [58, 59]. Herbal medicines, food supplements, and phytotherapy approaches provide a plethora of bioactive molecules and entire phytocomplexes potentially useful for mood disorders [60, 61]. Saffron and its main bioactive components crocin and safranal are promising candidates. Saffron has demonstrated antidepressant effects in clinical studies alone or associated with other natural compounds such as curcumin [25, 62].

Saffron's antidepressant properties have been extensively analyzed in clinical trials versus placebo [25, 63–66] or standard psychiatric drugs [67] such as fluoxetine [68–71], imipramine [72], citalopram [73], and sertraline [74]. Crocin, the main bioactive molecule of saffron, was also studied as an adjunctive treatment for depression [75].

In a 12-week double-blind, placebo-controlled trial, the effect of saffron on anxiety and depression was evaluated. Sixty adults with depression and anxiety randomly received a 50 mg saffron capsule or placebo twice daily for 12 weeks.

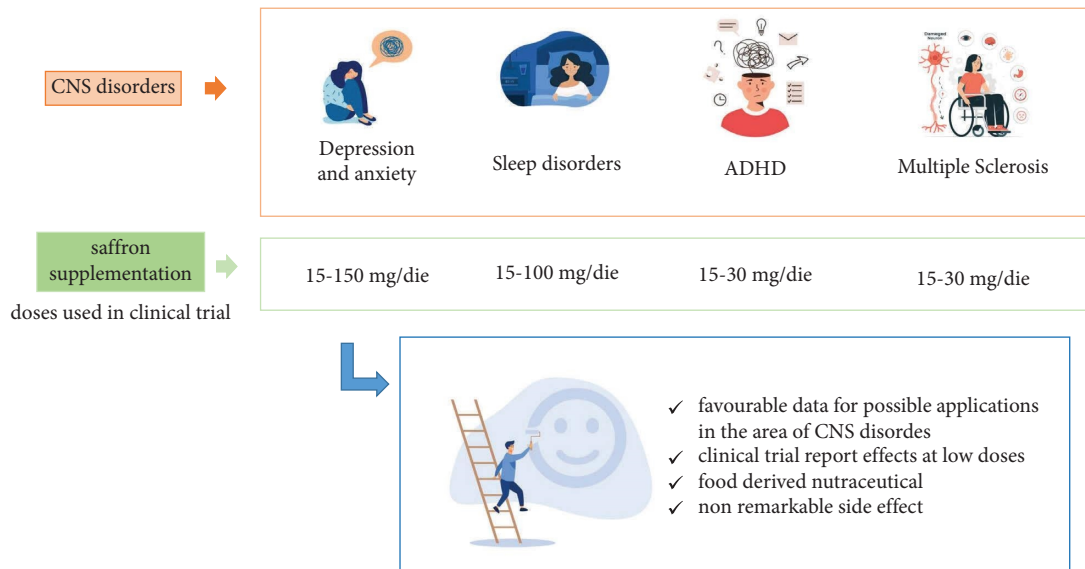


FIGURE 2: Chart of CNS disorders investigated with supplementation of saffron, doses used in clinical trials, and positive aspects of the treatment.

BDI and BAI scores were assessed at baseline and at 6 and 12 weeks. The results showed significant antidepressive and anxiolytic effects of saffron extract compared to placebo at 12 weeks with rare side effects [76].

Saffron's antidepressant and anxiolytic effects were also evaluated in 68 teenagers aged 12–16 years with mild to moderate anxiety or depression symptoms, in an 8-week, randomized, double-blind, placebo-controlled study. Teenagers in the saffron (Affron®, 14 mg b.i.d) group reported relevant improvements in depression, overall internalizing symptoms, social phobia, and separation anxiety analyzed with the Revised Child Anxiety and Depression Scale (RCADS). The authors highlighted the limitations of the study with the use of a self-report questionnaire, a single dose, and the parent's result showing no differences [77].

Interestingly, a recent study aimed to measure in untrained young males saffron's effect combined with six weeks of resistance training (RT) on neurotransmitters and biomarkers related to depression and happiness levels. Saffron (150 mg) was given immediately after each RT session and at the same time on nontraining days. The results showed a significant increase in 2-arachidonoylglycerol (2-AG), anandamide (AEA), dopamine, and β -endorphin in the saffron group, while serotonin levels and happiness scores increased in both groups as well as muscular endurance [78].

Since ancient times, on Santorini island, saffron was used as a remedy for depression and mood changes in women during the menstrual cycle, menopause, or postpartum [79]. In the last decade, many clinical trials have investigated saffron use for treating these women's conditions [30, 79–82]. In a recent study, sixty women presenting postmenopausal hot flashes received either saffron (30 mg/day; 15 mg twice per day) or a placebo for 6 weeks. The authors reported a significant improvement in hot flashes and depressive symptoms measured with the Hot Flash-Related Daily Interference Scale (HFRDIS) and the Hamilton Depression Rating Scale (HDRS). Saffron use

results as an effective and safe treatment in improving postmenopausal symptoms in healthy women [79].

Two main clinical trials have shown saffron effectiveness in mild to moderate postpartum depressive disorder. One double-blind, randomized, and placebo-controlled trial was performed on sixty new mothers with a maximum score of 29 on the Beck Depression Inventory-Second Edition (BDI-II). Patients received saffron (15 mg/b.i.d) or placebo treatment. Saffron treatment showed a more significant impact on the BDI-II scores (96% remission) than the placebo (43% remission) [80]. A second double-blind clinical trial compared the effectiveness and safety of saffron (15 mg capsule) or fluoxetine (20 mg capsule) in women aged 18–45 years with mild to moderate postpartum depression with an HDRS score of ≤ 18 . Saffron supplementation was a safe and effective alternative treatment for improving depressive symptoms of postpartum depression [68].

Saffron's antidepressive and antianxiety properties were confirmed by three relevant, recent meta-analyses. According to the first one, saffron showed significant antidepressive activity, more than placebo, and was non-inferior to standard antidepressant drugs [66]. The second meta-analysis in 2020, included twelve studies that compared the HAM-D or BDI scores, remission, response rate, and adverse effects between saffron and placebo or saffron and antidepressant drugs. Overall, results indicated a better improvement of depressive symptoms with saffron compared to placebo and the same effectiveness with antidepressant drugs [25].

In 2022, a relevant umbrella meta-analysis, including 7 meta-analyses, was carried out to fully understand the evidence of saffron's effectiveness in depression. Saffron use significantly decreased the BDI scores, with no change in the HAM-D scores and mixed scores (HAM-D/BDI/DASS) [83]. Overall, saffron might alleviate depression symptoms, but it cannot be a single therapeutic option to treat depression [64].

Based on the abovementioned meta-analysis and clinical trials, a minimum of 4–6 weeks of saffron treatment is required for antidepressant and anti-anxiety effects, with a daily dose ranging between 28 and 150 mg. Saffron might represent an alternative to standard antidepressants in the treatment of early symptoms of depression and anxiety. In combination with standard therapy, it seems to be a promising approach to treating mild to moderate depression. However, multicenter trials with longer treatment duration, larger sample sizes, and crossover, as the third phase of clinical trials, are necessary for a better understanding of saffron effectiveness in depression and anxiety. Furthermore, the use of standardized preparations with a precise amount of active constituents will be of help in understanding the relevance of saffron extracts in this therapeutic area.

3.3.2. Sleep Disorders. Sleep disorders are common complaints affecting human performance and may be experienced at different ages, in many cases becoming a chronic condition impacting patient's daily life. Sleep disorders can also be a symptom of other disorders, for example, depression, and frequently consist of repeated awakenings during the nighttime, prolonged wakefulness during the sleep period, long sleep latency, and transient arousals with reduced sleep quality. With pharmacological therapies, herbal remedies are commonly used as complementary and alternative insomnia treatments. In particular, saffron, crocin and safranal, has shown relevant hypnotic effects due to the inhibition of the wakefulness-promoting neurons in the tuberomammillary nucleus and the simultaneous activation of the sleep-promoting neurons in the ventrolateral preoptic nucleus. Different clinical trials and two recent meta-analyses have highlighted saffron's hypnotic properties [27, 28, 84–86] and one clinical trial for saffron's bioactive molecule crocetin [87]. In a 28-day, 3-arm, parallel-group, double-blind, randomized controlled trial, the effect of 14 mg, or 28 mg of a standardized saffron extract (Affron®) 1 h before bed, on 120 adults with unsatisfactory sleep versus placebo was measured using the Insomnia Symptom Questionnaire (ISQ), the Pittsburgh Sleep Diary, Restorative Sleep Questionnaire, Profile of Mood States, the Functional Outcomes of Sleep Questionnaire, and evening salivary cortisol and melatonin levels [27]. Saffron supplementation induced relevant improvements in sleep quality ratings, ISQ total score, and ISQ-insomnia classifications and mood ratings after awakening with no differences in the other questionnaires and outcomes. Moreover, saffron supplementation induced an increase in evening melatonin concentrations without affecting cortisol levels. No adverse side effects were reported [27].

A recent meta-analysis including eight articles with a total of 431 participants has shown a reduction of insomnia and increased sleep quality and duration with the saffron treatment compared to placebo groups [28]. Another meta-analysis run by Lian et al. in 2022 [84] compared the effect of different saffron dosages on sleep quality among healthy adults, patients with insomnia or type 2 diabetes, and patients under methadone maintenance treatment. Saffron

supplementation (100 mg/day) improved sleep quality in the subgroup analysis compared to placebo according to the Pittsburgh Sleep Quality Index (PSQI), the Restorative Sleep Questionnaire (RSQ), and the Insomnia Severity Index (ISI).

Overall, even though evidence of a moderate-to-high quality is still limited, saffron supplementation benefits people with insomnia and may reduce the use of sedative drugs, dependency, and withdrawal symptoms. Furthermore, RCT and meta-analysis with larger sample size and long duration are required to consolidate saffron benefits in diagnosed insomnia and other psychogenic and demographic characteristics and to unveil its potential sleep-enhancing mechanisms of action.

3.3.3. Attention-Deficit/Hyperactivity Disorder (ADHD). ADHD is a common neuropsychiatric disorder in childhood and adolescence. More than 30% of patients do not respond appropriately to standard therapy or cannot tolerate its side effects. Thus, alternative treatments, such as herbal medicine, are under investigation [88]. Saffron extract has been evaluated in at least five clinical trials for its effectiveness in ADHD compared to standard therapy methylphenidate (MPH) or in combination with MPH [55].

A randomized double-blind 6-week study considered 54 children (aged 6–17 years) with a diagnosis of ADHD. Patients received either MPH (20–30 mg/die) or saffron (20–30 mg/die) randomly. Results revealed no significant changes in teacher and parent ADHD Rating Scale scores between the saffron group and the MPH group, suggesting that short-term saffron supplementation can have the same efficacy as MPH treatment [89].

Two studies evaluated the effectiveness of MPH in combination with saffron (20–30 mg/day) in children with ADHD: in the first study, 70 children aged between 6 and 16 years diagnosed with ADHD were treated with a combination therapy of MPH and saffron and resulted in more effectiveness compared to separate treatments [90]. In a second randomized, double-blind, placebo-controlled clinical trial, fifty-six patients with ADHD received Ritalin® (30 mg/day) plus placebo or Ritalin® plus saffron (15 mg twice daily) for six weeks. When saffron was added to therapy with Ritalin, symptoms were effectively improved [29].

A recent clinical trial investigated the effect of psychoeducation and saffron (30 mg/day) versus psychoeducation and extended-release MPH in 63 children aged 7–17 with ADHD for 3 months. Children and their parents could choose their treatment group. Results showed comparable efficacy of saffron to that of MPH. Interestingly, saffron was more effective in hyperactivity symptoms, while MPH was more effective in inattention symptoms [91].

Despite saffron seeming a promising natural medicine for ADHD, further RCT and meta-analysis with larger size samples and long duration are required.

3.3.4. Multiple Sclerosis. Multiple sclerosis (MS) is a debilitating autoimmune disease resulting in progressive demyelination and neurodegeneration. Excessive oxidative stress, inflammation, and DNA damage have been observed

in MS patients together with fatigue and mental and psychological discomfort. Due to its anti-inflammatory and antioxidant capacity and antidepressive and cognitive-enhancing properties, saffron supplementation has recently been investigated as a complementary adjunct supplementation in multiple sclerosis (MS).

A prepost study evaluated the consumption of 7.5 cc every 8h for two months of saffron syrup to relieve the fatigue of 30 MS patients according to the fatigue severity scale (FSS). Results showed promising antifatigue effects of simple saffron syrup in patients with MS with no significant side effects [92].

Patients with MS often possess a higher level of oxidative stress, inflammatory markers, and DNA damage than healthy subjects [93, 94]. In one study, the effect of oral crocin on oxidative stress in MS patients in addition to standard treatment for 4 weeks was tested. The crocin group (15 mg twice daily) resulted in a significant increase in total antioxidant capacity levels, saliva total thiol groups, and catalase activity and a marked decrease in lipid peroxidation level. According to the study, crocin can reduce several oxidative stress factors in MS patients [95]. Another study aimed to evaluate the effect of crocin on oxidative damage, inflammatory markers, and DNA damage in the blood of MS patients. 40 patients were randomly divided into two groups where the intervention groups received two crocin capsules per day for 28 days. A significant reduction in DNA damage, lipid peroxidation, tumor necrosis factor- α , and interleukin-17 and a significant increase in the total antioxidant capacity were measured in the serum of MS patients treated with crocin, suggesting a beneficial effect of crocin in MS patients [96].

In MS patients, matrix metalloproteinase-9 (MMP-9) facilitates T-cell migration to the CNS, while tissue inhibitors of metalloproteinase-1 (TIMP-1) decrease MMP-9 activity [97]. Saffron supplementation in relapsing-remitting MS patients for 12 months noticeably decreased MMP-9 serum levels in patients with MS and significantly increased the levels of TIMP-1. No differences were observed with the placebo [98].

A recent systematic review of randomized controlled trials (RCTs) comparing saffron supplementation to placebo, or other interventions, in patients with an MS diagnosis, concluded that saffron supplementation might improve antioxidant defense and reduce excessive oxidative stress in MS patients. The strength of the evidence is limited, scattered, and inadequate, and further better-designed trials with MS-specific outcomes are required [26].

3.3.5. The Use of Saffron as Nutraceutical for Preventive and Protective Activities on CNS. As seen so far, different pre-clinical and clinical trials have revealed the potential usefulness of saffron in different CNS pathological manifestations. As shown in Table 3, doses used in many experiments with patients are relatively low, ranging from 15 to 150 mg/die. Literature indicates that at those doses that are considered therapeutic, no significant toxicity is reported [2, 6, 33]. The large amount of preclinical and clinical data

showing the potential usefulness and low toxicity suggest the potential use of saffron in large part of population ranging from children to elderly people. The relevant role of saffron constituents on oxidative stress and mitochondrial metabolism underlines the basis of its possible application as a preventive and protecting agent especially related to CNS due to its sensitivity to oxidative stress. Several studies have shown a significant role of saffron as an active agent in Parkinson's disease [5, 22] and in some models of Alzheimer's disease [20, 24, 43], and thus, it can be presumable that its supplementation in healthy subjects can be protective to control ROS-related degenerative processes, supporting its role as a nutraceutical.

Due to the need to administrate sufficient doses of bioactive saffron constituents, attention should be paid towards levels of saffron compounds and their bioavailability when used in products. Nowadays, standardization and analysis of different classes of saffron constituents can be performed with great attention and confidence using several analytical approaches. Nevertheless, in the market products, saffron can be present both with optimal levels of phytoconstituents or without any precise determination. Therefore, to establish the quality of saffron supplements, the chemical constituents should be kept in mind.

Many research studies have been performed in the area of saffron considering the various extraction procedures ranging from the more traditional going to the more sustainable and technologically driven [99, 100]. More recent papers underlined the importance of plant material selection and the standardization of the active constituents in the starting materials to ensure the accurate amount in the final formulated product [101]. Up to now, limited information is available related to the product used in the food supplement or nutraceutical area, and more research studies are needed both in the analytical approach and in the whole processing of the product to ensure accurate labeling. Up to now, the published literature allows us to consider the average doses that we reported in Table 3 and in the further chapters as schematized in Figure 3. The new challenge for the future application of saffron will be the gap-filling between the observed clinical effects and the "active" constituents that can be extracted or formulated in various forms.

3.4. Bioavailability of Main Saffron Constituents. The efficacy of a nutraceutical product containing saffron and its specific activity on the CNS system imply the absorption distribution and metabolism of the phytochemicals involved in the bioactivities. The study of the bioavailability of natural products is extremely challenging because plant extracts are composed by different chemicals and can be contained in different matrices. Plant-derived products are chemically different and present distinct solubilities. In addition, they can be modified by host and microbial metabolism, and their plasmatic levels can be altered by the presence of other compounds such as saponins, polysaccharides, or proteins. Saffron apocarotenoids have been studied, and some papers described pharmacokinetics and bioaccessibility. In 2018, a review summarized the main information about the

TABLE 3: Studies involving the use of saffron or saffron extracts in depression.

| First author | Group | Daily dose | Sample size | Duration | Outcomes |
|------------------------------|--|--|---------------------------------|----------|--|
| Mazidi et al. 2016 [76] | Saffron vs placebo | 50 mg b.i.d saffron | 60 adults | 12 weeks | Reduction in BDI and BAI scores with saffron |
| Lopresti et al. 2018 [77] | Affron® vs placebo | 14 mg b.i.d saffron | 68 teenagers | 8 weeks | Improvements in overall internalizing symptoms, social phobia, depression, and separation anxiety analyzed with RCADS with saffron |
| Kell et al. 2017 [64] | Affron® vs placebo | 28 mg or 22 mg saffron | 128 adults | 4 weeks | Reduction in negative mood, stress, and anxiety only with 28 mg/day measured with POMPS and PANAS questionnaire, DASS-21 scale, and PSQI |
| Moghadam et al. 2021 [78] | Saffron + RT vs RT + placebo | 150 mg saffron | 28 untrained young male | 6 weeks | Increase in AEA, 2-AG, dopamine, and beta-endorphin in saffron + RT; increase in serotonin, happiness, and muscular endurance in both groups with a greater increase in the saffron + RT group |
| Kashani et al. 2018 [79] | Saffron vs placebo | 15 mg b.i.d saffron | 60 postmenopausal women | 6 weeks | Improvements in hot flashes and depressive symptoms measured with HFRDIS and HDRS |
| Tabeshpour et al. 2017 [80] | Saffron vs placebo | 15 mg b.i.d saffron | 60 new mothers with BDI-II < 29 | 8 weeks | Higher reduction in BDI-II scores of saffron; remission in 93% of the saffron group and 43% of the placebo group |
| Sahraian et al. 2016 [70] | Saffron + fluoxetine vs fluoxetine + placebo | 30 mg saffron 20 mg fluoxetine | 40 adults | 4 weeks | Improvements in depression severity in both the groups without significant differences |
| Talaei et al. 2014 [75] | Crocin + SSRI vs SSRI + placebo | 15 mg b.i.d crocin 20 mg fluoxetine or 50 mg sertraline or 20 mg citalopram | 40 MDD adults | 4 weeks | Higher reduction in BDI, BAI, and GHQ scores in the saffron group + SSRI |
| Akhondzadeh et al. 2020 [63] | Saffron vs placebo | 15 mg b.i.d saffron | 73 MMD women with BMI ≥ 25 | 12 weeks | Reduction in BDI-II in the saffron group. No effect on food craving |

TABLE 3: Continued.

| First author | Group | Daily dose | Sample size | Duration | Outcomes |
|-----------------------------|---|---|---|----------|---|
| Lopresti et al. 2019 [67] | Affron® vs placebo adjunct to standard antidepressant therapy | 14 mg b.i.d saffron | 160 adults with persistent depression | 8 weeks | Higher reduction in MADRS in the saffron group. No difference in MADRS-S |
| Kashani et al. 2017 [68] | Saffron vs fluoxetine | 15 mg b.i.d saffron 20 mg b.i.d fluoxetine | 94 adult women with postpartum depression | 6 weeks | Significant improvements in HDRS scores in both groups No significant difference between saffron and fluoxetine |
| Ghajar et al. 2017 [73] | Saffron vs citalopram | 30 mg saffron 40 mg citalopram | 66 MDD adults with anxious distress | 6 weeks | Significant improvements in HAM-D and HAM-A scores in both groups No significant difference between saffron and citalopram |
| Ahmadpanah et al. 2019 [74] | Saffron vs sertraline | 60 mg saffron 100 mg sertraline | 50 older out-patients with MDD | 6 weeks | Significant improvements in HDRS in both groups No significant difference between saffron and sertraline |

BDI, Beck Depression Inventory; BAI, Beck Anxiety Inventory; MDD, major depressive disorder; HAM-D, Hamilton Depression Rating Scale; HAM-A, Hamilton Anxiety Rating Scale; HDRS, Hamilton Depression Rating Scale; MADRS, Montgomery-Asberg Depression Rating Scale; GHQ, General Health Questionnaire; SSRI, selective serotonin reuptake inhibitor; AEA, arachidonyl ethanolamine; 2-AG, arachidonyl glycerol; RCADS, Revised Children's Anxiety and Depression Scale; POMPS, primary outcome measure; DASS-21, Depression Anxiety Stress Scale; PSQI, Pittsburgh Sleep Quality Index; HFRDIS, Hot Flashes-Related Daily Interference Scale.

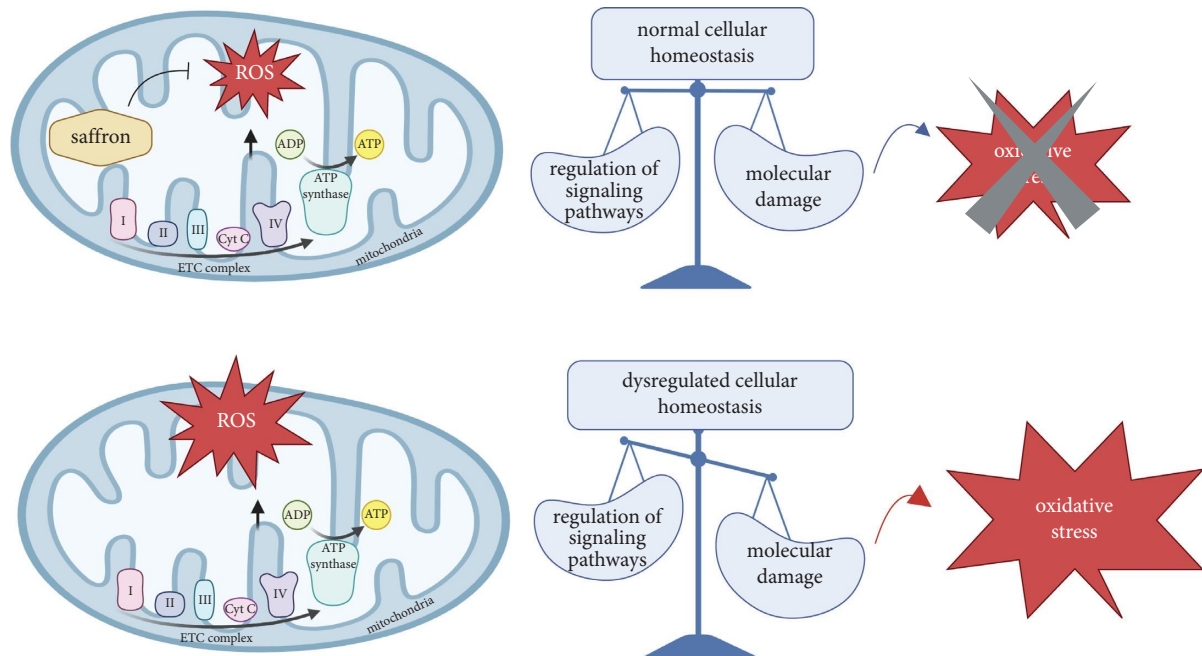


FIGURE 3: ROS is considered signaling molecules and dangerous elements that affect normal cellular homeostasis. Therefore, a balance between their actions as regulatory molecules of signaling pathways and damaging signals should be maintained to sustain cellular homeostasis. However, during stress conditions, the equilibrium shifts toward increased oxidative stress. With its antioxidant capacity, saffron can restore normal cellular homeostasis.

pharmacokinetic properties of saffron and its constituents [102]. More recently, other authors evaluated the bioaccessibility of saffron constituents or at least their plasma levels after the administration of commercial extracts [16, 32, 36, 103].

Some papers considered the carotenoids of saffron and their pharmacokinetics or absorption after oral administration. Results have been reviewed by Hosseini et al. in 2018 [102] and more recently by Song et al. [104]. Crocin and its derivatives contain ester linkage with sugars and crocin-I had a strong water solubility and weak lipophilicity and was not easily absorbed by oral administration [104]. Furthermore, after oral administration, at least a part of this ester is cleaved [36, 104] by the acidic medium of gastric fluid or by enzymatic activities present in the gastrointestinal tract. Crocin bioavailability has been extensively studied measuring the crocetin plasmatic levels due to this modification [102]. Thanks to its lower molecular weight and the absence of sugar residues, crocetin is more easily absorbed than crocin. Indeed, crocetin is rapidly eliminated from plasma. Most of the studies published so far related to the bioaccessibility and bioavailability of saffron constituents were mostly conducted in *in vitro* experiments, cell models, and some cases animal tests [36, 104]. Crocetin penetrated the intestinal tract through passive transcellular transport without specific carriers as transcrocetin [104]. Studies performed in animal models indicated that after oral dosing of crocins or crocetin, only crocetin can be measured in the rat and mouse plasma in the form of free glucuronide, monoglucuronide, and diglucuronide

conjugates [2, 36, 104]. Thus, crocins are highly metabolized and converted into smaller and more lipophilic crocetin. Furthermore, when crocin is administered intravenously, lower crocetin concentration is observed in plasma, suggesting that crocin is converted into crocetin mainly in the gastrointestinal tract [102]. In 2018, the penetration of trans-4 crocin in mice brains after intraperitoneal injection was reported [105] confirming that the transformation of crocin into crocetin occurs only in the GI tract. A schematic representation of the crocetin absorption is represented in Figure 4.

Recently, a lyophilized extract mostly containing transcrocetin was used to assess the pharmacokinetics of crocetin and its metabolite after the administration of a single dose (i.v. and oral) of saffron extract at 60 mg/kg of body weight to male mice (C57Bl/6J). The authors reported that the kinetic can be described by a one-compartment PK distribution model and crocetin's oral absorption can be described by first-order kinetic constant.

Serum and tissue noncompartmental pharmacokinetic analysis revealed a relative oral bioavailability for total crocetin that was calculated to be 1.17 times the administered crocin. This is caused by the modification of the different crocin derivatives into crocetin. Furthermore, the authors observed extensive crocetin distribution to the liver and kidneys [32]. The pharmacokinetics of these compounds are complicated because the crocin present in aqueous saffron extract is subjected to a rapid hydrolysis in the gastrointestinal tract leading to crocetin formation. This latter compound is extensively absorbed as demonstrated by the measured serum and tissue crocetin and crocetin levels

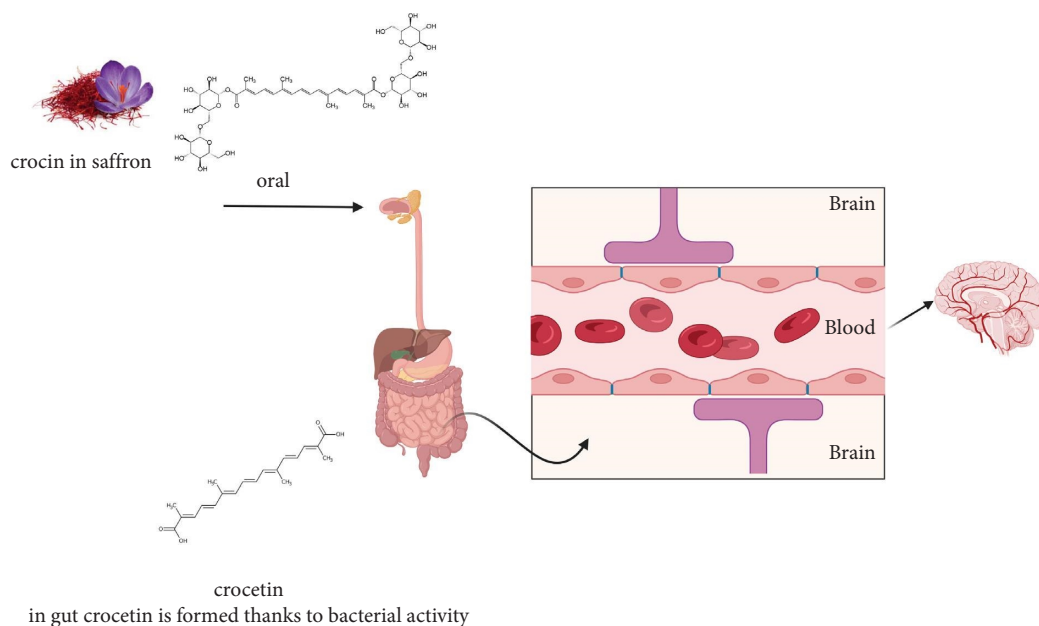


FIGURE 4: Schematic representation of the formation of crocetin in the GI tract and its absorption.

after oral administration of the saffron extract (60 mg/kg). The total crocetin serum AUC values were 1.5–2.5 times higher than those measured for crocetin reflecting crocetin glucuronidation [32].

A key point for the evaluation of the saffron constituent's bioavailability is to use standardized extracts. In recent years, some works have proposed pharmacokinetic studies on specific extracts. Affron[®], a standardized extract, has been used to study the effect of *in vitro* digestion on the main bioactive components of saffron extract tablets and to evaluate the pharmacokinetic parameters in humans after the administration of the product to thirteen healthy volunteers [36]. The extract contains 3.6% of total crocins, 3.2% of picrocrocin, 0.13% of kaempferol diglucoside, and 0.04% of safranal. The *in vitro* digestion induced an increase in the safranal content (with 200% bioaccessibility) and a decrease in the picrocrocin content (81% bioaccessibility) due to the hydrolytic processes and a reduction in kaempferol diglucoside (70% bioaccessibility) [36]. In the plasma of volunteers receiving 56 mg or 84 mg of affron, crocins, picrocrocin, and safranal was not detected. Crocetin was detected, and its concentration in plasma grew up to 60 and 90 min, respectively, and then gradually decreased [36]. The same authors reported that a standardized extract containing crocetin induces the same T_{max} of crocin when compared to previous studies in which purified crocin was administered. In addition, crocins, thanks to the sugar moieties, are probably more bioavailable to enterocytes for later hydrolysis and absorption than the arrival of crocetin itself [36].

Other papers described saffron's constituent bioavailability after an oral administration to 10 healthy volunteers receiving 300 mg of the saffron extract. The extract used was a standardized product containing crocins (mainly

trans-4-GG, trans-3-Gg; cis-4-GG, and trans-2-G) >3%, safranal >0.2%, picrocrocin derivatives (mainly picrocrocin and HTCC) >1%, and kaempferol derivatives (mainly kaempferol-3-sophoroside-7-glucoside and kaempferol-3-sophoroside) >0.1%, measured by the UHPLC method. Crocetin was used as a validated marker of saffron extract absorption. The absorption profile showed that the maximum crocetin concentration was reached 90 min after ingestion [103]. No information was reported related to other constituents of the extract. Up to now, studies considering the pharmacokinetics of different compounds are still lacking, and further research is needed to clarify the role of flavonoids and other constituents beyond crocins able to influence the bioavailability of active saffron compounds.

4. Conclusion

Saffron, the “red gold,” is a spice used and known in several parts of the world and is largely present in cooking traditions as well as used as a traditional remedy. Many studies have considered this spice for its potential bioactivities showing relevant antioxidant and anti-inflammatory activities using different *in vitro* models showing potential usefulness for the CNS system, especially considering the crucial role of oxidative stress in different pathological conditions of the brain. Due to the low toxicity of saffron and its constituents, there is a renewed attention to this spice as a potential nutraceutical ingredient targeted on CNS. In particular, several studies have shown significant effects in depression, ADHD, multiple sclerosis, and sleep disorders.

The new challenge for a more effective and safe use of this nutraceutical will be a more deep investigation on the different chemical constituents and their role in the observed effects, thus the need of accurate standardization of the product. Furthermore, the role of such products as

protective remedies is able to reduce at least in some parts of the population the onset of pathological conditions such as insomnia or depression offering a nutritional or nutraceutical valuable approach.

Data Availability

No data were used to support the study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

S1: saffron chemical constituents: apocarotenoids and carotenoids. S2: saffron chemical constituents: flavonoids and other constituents. Figure S1: main-colored compounds in saffron. Figure S2: structures of crocrocetin and its degradation product safranal. (*Supplementary Materials*)

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