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REVIEW ARTICLE

Antioxidant Properties of *Crocus Sativus* L. and Its Constituents and Relevance to Neurodegenerative Diseases; Focus on Alzheimer's and Parkinson's Disease

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Abstract: ***Background*:** Reactive oxygen species and reactive nitrogen species, which are collectively called reactive oxygen-nitrogen species, are the inevitable by-products of cellular metabolic redox reactions, such as oxidative phosphorylation in the mitochondrial respiratory chain, phagocytosis, reactions of biotransformation of exogenous and endogenous substrata in endoplasmic reticulum, eicosanoid synthesis, and redox reactions in the presence of metal with variable valence. Among medicinal plants, there is growing interest in *Crocus Sativus* L. It is a perennial, stemless herb, belonging to *Iridaceae* family, cultivated in various countries such as Greece, Italy, Spain, Israel, Morocco, Turkey, Iran, India, China, Egypt and Mexico.

***Objective*:** The present study aims to address the protective role of *Crocus Sativus* L. in neurodegeneration with an emphasis in Parkinson's and Alzheimer's disease.

***Materials and Methods*:** An electronic literature search was conducted by two of the authors from 1993 to August 2017. Original articles and systematic reviews (with or without meta-analysis), as well as case reports were selected. Titles and abstracts of papers were screened by a third reviewer to determine whether they met the eligibility criteria, and full texts of the selected articles were retrieved.

***Results*:** Hence, the authors focused on the literature concerning the role of *Crocus Sativus* L. on its anti-oxidant and neuroprotective properties.

***Conclusion*:** Literature findings represented in current review herald promising results for using *Crocus Sativus* L. and/or its active constituents as antioxidants, anti-inflammatory, and neuroprotective agents.

Keywords: Saffron, crocin, crocetin, safranal, oxidative stress, neurodegeneration, Alzheimer's disease, Parkinson's disease.

1. INTRODUCTION

1.1. Reactive Oxygen Species and Reactive Nitrogen Species

Reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are collectively called reactive oxygen nitrogen species (RONS), comprise the inevitable by-products of cellular metabolic redox reactions, such as oxidative phosphorylation, phagocytosis, transformation of exogenous and endogenous substrates in endoplasmic reticulum (ER), synthesis of eicosanoids, and oxidation reactions in the presence of variable -valence metals. Hydroxyl (OH•), superoxide anion (O_2•$^-$), oxygen radical (O_2••), peroxyl (RO_2•), alkoxyl (RO•), lipid peroxyl (LOO•), nitric oxide (NO•), and nitrogen dioxide (NO_2•) are the most common ROS and RNS molecules. Additional oxygen and nitrogen by-products, such as hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$), ozone (O_3), hypochlorous acid (HOCl), hypobromous acid (HOBr), nitrous acid (HNO_2), nitroxyl anion (NO^-), nitrosyl cation (NO^+), dinitrogen tetraoxide (N_2O_4), dinitrogen trioxide (N_2O_3), nitryl cation (NO_2^+), peroxynitrite (ONOO•),

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Table 1. List of RONS.

Name	Symbol
Hydroxyl	OH•
Superoxide ion	$O2^{\bullet -}$
Oxygen radical	$O_2^{\bullet\bullet}$
Hydrogen peroxide	H_2O_2
Singlet oxygen	$^1O_2$
Hypochlorous acid	HOCl
Nitrous acid	HNO_2
Peroxynitrite	ONOO•
Nitrite	NO_2^-
Nitrate	NO_3^{--}
Nitric oxide	NO•
Nitrogen dioxide	NO_2•
Peroxyl radical	RO_2•
Alkoxyl radical	RO-
Lipid peroxyl radical	LOO•
S-nitrosothiol	RSNO

organic peroxides (ROOH), and aldehydes (HCOR) are mediators of free radical reactions, as they are easily converted into radicals or act as oxidizing agents (Table **1**).

Under normal metabolic conditions, complex III ($CoQH_2$-cytochrome c reductase/cytochrome bc_1 complex) and to a lesser extent complex I (NADH: ubiquinone oxidoreductase/NADH-dehydrogenase) of mitochondrial electron-transport chain are the main sites of $O_2^{\bullet -}$ generation. The $O_2^{\bullet -}$ is produced in a non-enzymatic way, thus the production of ROS is proportional to the cellular metabolic rate.

In peroxisomes, during cellular respiration, electron transfer from different metabolites to oxygen or b-oxidation of fatty acids leads to H_2O_2 formation. Other ROS which are produced in peroxisomes are: OH•, $O_2^{\bullet -}$, and NO•. In peroxisomes, the enzymes contributing to ROS generation are D-amino acid oxidase, acyl CoA oxidase, L-a-hydroxy oxidase, xanthine oxidase and urate oxidase. The enzymes of ER implicated in the formation of ROS include diamine oxidase, cytochrome p-450 and b5 enzymes, and a thiol oxidase enzyme, Erop1p. Other enzymes implicated in ROS generation are the membrane-bound enzymes NADPH (nicotinamide adenine dinucleotide phosphate) oxidase and cyclooxygenase (COX), the nitric oxide synthase (NOS) and lipoxygenase (LOX), whose intracellular compartmentalization differs in the cells

Superoxide anion radical, the product of one-electron reduction of oxygen, is the most important and abundant ROS, as it is the precursor of most other ROS. It is formed by enzymatic processes or non-enzymatic electron transfer reactions and autoxidation. It is mostly produced within the mitochondria and or in the presence of xanthine oxidase, LOX, COX and NADPH dependent oxidase. At low pH, it can exist as perhydroxyl radical (HOO•), which is the simplest form of peroxyl radical and can easily enter the phospholipid bilayers, initiating lipid peroxidation. However, its reactivity with cellular molecules is low. Superoxide anion acts as a reducing agent, reducing iron complexes, *e.g*. cytochrome-c, in which Fe^{+3} is reduced to Fe^{+2}, as well as an oxidizing agent, *e.g*. oxidizing tocopherol and ascorbic acid.

Hydrogen peroxide is generated spontaneously or *via* a reaction catalysed by SOD (dismutation). Although it is not an ROS, it can easily penetrate biological membranes, causing cellular damage and inactivating enzymes implicated in cellular energy production, such as glyceraldehyde-3-phosphate dehydrogenase. It can be fully reduced to water, whereas in the presence of transition metal ions (ferrous or cuprous ions), it produces OH•.

Hydroxyl radical is a powerful oxidant, causing severe cellular damage. It is generated from H_2O_2, when it reacts with metal ions (Fe^{+2} or Cu^+), often bound to proteins, *e.g*. ferritin and ceruloplasmin, producing in parallel Fe^{+3} or Cu^{+2} in *Fenton* reaction. Under stress conditions, excess $O_2^{\bullet -}$ can react with H_2O_2 to yield OH• (Haber-Weiss reaction).

Nitric oxide is a small, short-lived molecule, formed from L-arginine by different tissue-specific NOS, in the presence of NADPH (nicotinamide-adenine-dinucleotide phosphate) and O_2, producing at the same time L-citrulline and NADP. The molecule is both lipophilic and hydrophilic, hence readily diffusing *via* cytoplasm and plasma membranes. There are three isoforms of NOS: the constitutive isoforms nNOS (neuronal NOS) and eNOS (endothelial NOS) found in neuronal and vascular endothelial cells, respectively and iNOS (inducible NOS) in microglia and endothelial cells, hepatocytes, keratinocytes and macrophages. nNOS and eNOS are activated in response to physiological stimuli and are Ca^{2+}-dependent; an increase in intracellular Ca^{2+} increases the affinity of calmodulin to NOS, facilitating electron flow from NADPH in the carboxy-terminal reductase domain to the heme in the amino-terminal oxygenase domain. NO is an important intracellular second messenger, stimulating guanylate cyclase and regulating enzymatic activity by nitrosylating protein kinases, thus implicated in the vasodilatory tone, tissue perfusion, platelet adhesion, cellular proliferation, gene transcription, mRNA translation (*e.g*. binding to iron-responsive elements), post-translational protein modifications (*e.g*. ADP ribosylation), neurotransmission, memory formation, immune regulation, and cellular redox regulation, acting both as an antioxidant and a free radical.

Singlet oxygen is a highly reactive toxic ROS, produced by activated neutrophils and eosinophils and any other cell during enzymatic reactions catalysed by LOX, dioxygenases, and lactoperoxidase. Ozone is also an important oxidant, produced during inflammation *via* the antibody-catalysed water oxidation pathway. Hypochlorous acid is a strong ROS, produced from H_2O_2 and chloride in a reaction catalysed by myeloperoxidase. It is mainly formed at inflammatory sites by activated neutrophils.

Peroxynitrite is highly toxic, generated by the reaction between NO• and $O_2•^-$, in a reaction controlled by the rate of diffusion of both radicals. It directly reacts with CO_2, forming the highly reactive peroxynitrous acid (ONOOH) or nitroso peroxo carboxylate ($ONOOCO_2^-$). Peroxynitrous acid can undergo proton-catalysed homolysis to yield OH• and NO_2 or rearrange to NO_3^-. The nitrotyrosine residues could serve as indicators of peroxynitrite-induced cellular damage [1-9].

The generation of RONS is a natural consequence of aerobic metabolism and is integral for maintaining tissue oxygen homeostasis. In this regard, ROS are not simply a pernicious product of a defective system, but at moderate concentrations, are implicated in core physiologic processes, such as vasodilation, synaptic plasticity, clotting, effective immune defence and glucose uptake by skeletal muscles. Also, RONS can act as secondary messengers in intracellular pathways, inducing cell senescence and apoptosis, whereas low levels of ROS may favour cell proliferation, differentiation and migration. Thus, 1- 5% of oxygen cellular consumption is diverted to ROS production, but under normal conditions, the cellular redox state is maintained within a narrow range [7, 10-12].

Oxidative stress arises due to disturbed equilibrium between ROS generation and elimination or neutralization processes, in favour of the former. Overproduction of ROS is related to mitochondrial dysfunction and inactivation of respiratory-chain enzymes, respiratory burst and augmented the activity of various oxidases during environmental stress or inflammatory stimulus and/or exhaustion or impairment of cell's antioxidant capabilities.

1.2. Effects of RONS

RONS have exceptional chemical instability and are highly reactive, as they possess one or more unpaired electrons in the valence shell or outer orbit or non-static bonds, thus entering chemical reactions with other RONS and non-radical molecules (proteins, lipids, carbohydrates and DNA) to attain stability. Electron loss in the attacked molecule renders it a free radical, instigating a cascade with deleterious cellular effects. Also, oxidation of macromolecules alters their function and irreversibly destroys them, *e.g.* loss of enzymatic activities, DNA damage, inhibition of protein synthesis, and deregulation of ion transport.

1.2.1. Targeting DNA and RNA

Radicals can react with any component of DNA molecules, including the deoxyribose backbone, pyrimidine and purine bases, permanently modifying the genome. Mitochondrial DNA (mtDNA) is particularly susceptible to ROS attack, as it is located proximal to the ROS generating pool. Hydrogen abstraction from 1'-deoxyribose carbon of deoxyribose sugar backbone initiates a hydroxyl radical reaction, creating the 1'-deoxyribosyl radical. The latter reacts with oxygen, yielding peroxyl radicals, which subsequently are reduced and dehydrated to 2'-deoxyribonolactones and free bases. The major ROS-generated adducts of DNA's sugar moiety are 2-deoxytetrodialdose, 2-deoxypentonic acid lactone, erythrose, 2-deoxypentose-4-ulose, and glycolic acid. Hydroxyl radical reactions with DNA bases are mediated *via* interaction with the electron-rich, pi bonds, which are located between N7-C8 in purines and C5-C6 of pyrimidines. Pyrimidine adducts from OH• attack include uracil glycol, thymine glycol, 5-hydroxy deoxycytidine, and 5-hydroxydeoxy uridine (5-OHU), whereas purine adduct is 8-hydroxydeoxy guanosine (8-OHdG), which serves as a biomarker of oxidative DNA damage, 8-hydroxydeoxy adenosine (8-OHA), 4,6- diamino-5-formamidopyrimidine and 2,6-diamino-4-hydroxy-5-formamidopyrimidine. Other ROS- induced adducts of DNA bases include cytosine glycol, 5-formyl uracil, 5-hydroxylysine, 5-hydroxy-6-hydrocytosine, 5-hydroxy-6-hydro uracil, 5,6-dihydrothyronine, and alloxan.

Peroxynitrite can interact with guanine to form 8-oxodeoxyguanosine and 8-nitroguanine, which are unstable and can be removed spontaneously (apurininc site).

Ribonucleic acid (RNA) is more vulnerable to oxidative damage than DNA, as it possesses a single strand; it lacks active repair mechanisms or protective proteins and is located in close proximity to mitochondria. A common oxidative RNA damage product is 7, 8-dihydro-8-oxoguanosine (8-oxoG) [4, 5, 8, 13-18].

1.2.2. Lipid Peroxidation

Cellular membranes are particularly sensitive to oxidative alterations due to their physicochemical features, as they consist of bilayers of amphipathic molecules and the chemical reactivity of Poly-Unsaturated Fatty Acids (PUFAs) residues of phospholipids, the predominant membrane lipids, which are distributed across the bilayer in an asymmetric manner. Oxygen and ROS are particularly soluble in the lipid bilayer, where they have the tendency to concentrate, for triggering and maintaining a chain of oxidative reactions.

ROS react with the nucleophilic unsaturated double bonds of multiple, long-chain PUFAS residues of phospholipids, such as linoleic, arachidonic and docosahexanoic acids to generate lipid peroxidation products. Lipid peroxidation develops in three distinct stages: initiation, propagation, and termination. During the initiation step ROS attack methylene groups ($-CH_2$) of fatty acids and abstract hydrogen, resulting in the formation of lipid radicals (L•), which are relatively unstable. Lipid radicals react with molecular oxygen, forming at the propagation stage a lipid peroxyl radical (LOO•). The resultant LOO• is also highly reactive, attacking membrane proteins and oxidizing adjacent PUFAS, followed by the production of different fatty acid radicals and lipid peroxides, or cyclic peroxides, instigating a perpetual cascade from an initial attack. Termination occurs when two radicals react and produce a non-radical species or an antioxidant quenches the last radical.

Scission of oxidized PUFAs (hydroperoxides, endoperoxides) produces reactive α,β-unsaturated aldehydes [hydroxy-trans-2-nonenal (HNE), and 2-propenal acrolein], dialdehydes [malondialdehyde (MDA), and glyoxal] and ketoaldehydes [4-oxo-trans-2-nonenal (ONE) and isoketals], which mediate many harmful effects, associated with oxidative stress. These reactive aldehydes have a much longer half-life than most RONS, extending from minutes to hours, while their the non-charged structure allows them to dissolve

and migrate easily through hydrophobic membranes and the hydrophilic cytosol, thus attacking molecules located far from their production site. Hence, free radical-induced lipid peroxidation propagates and amplifies oxidant-mediated damage.

Acrolein, a ubiquitous environmental pollutant, is a hydrophilic, highly reactive unsaturated aldehyde, and also a by-product of endogenous lipid autoperoxidation, and polyamine metabolism and a toxic metabolite of cyclophosphamide. It remains as an intracellular cytotoxin, exerting high reactivity with cellular nucleophiles, such as DNA, proteins, and RNA. More specifically, susceptible amino acid residues are the imidazole group of histidine, the sulfhydryl group of cysteine, and the amino group of lysine, involved in major cellular mechanisms, *e.g*. ROS sensing, redox signaling, enzyme catalysis, and cellular buffering. Acrolein can react with DNA bases to produce cyclic adducts, the most abundant of which is the exocyclic adduct acrolein – deoxyguanosine, rendering acrolein highly mutagenic agent. Also, it inhibits DNA repair enzymes or slows DNA repair activity and is probably implicated in epigenetic alterations of gene expression by inhibiting histone acetylation. An intriguing feature is that acrolein acts both as a product and an initiator of oxidative stress, exerting its toxicity in a tissue- and disease-specific manner. Thus, it is implicated in several pathological conditions, such as Alzheimer's disease, multiple sclerosis, spinal cord injury, diabetes mellitus, cardiovascular diseases, neurotoxicity, hepatotoxicity, and nephrotoxicity. Acrolein interferes with its own metabolism by inhibiting alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) which are responsible for metabolizing acrolein-GSH conjugates.4-HNE is a diffusible, cytotoxic aldehyde, which forms covalent bonds with cysteine, histidine, and lysine, and nucleic acids (Michael addition). MDA is a mutagenic aldehyde, commonly used as a biomarker for lipid peroxidation, using the thiobarbituric acid reacting substance (TBARS) assay.

Reactive aldehydes can incorporate into amino acid residues, such as cysteine, proline, lysine, arginine and threonine to yield carbonyl-compounds, such as MDA-lysine, HNE-lysine, S-carboxymethyl–cysteine, and lysine–MDA–lysine. Carbonylated proteins are hydrophobic and resistant to proteolysis, which allows accumulation of non-functional proteins. The accumulation of MDA adducts on proteins is also involved in the generation of the non-degradable intralysosomal fluorescent pigment, lipofuscin. The carbonyl derivatives 3-nitrotyrosine (indicator of RNS), and O-tyrosine (indicator of OH•) are markers of ROS-mediated protein oxidation. Lipid peroxidation derivatives also react at the exocyclic amino groups of deoxy-guanosine, deoxy-adenosine, and deoxy-cytosine to produce alkylated products.

Lipid peroxidation alters directly the phospholipid asymmetry of the membrane lipid bilayer, leading to decreased fluidity and loss of membrane integrity. Oxidation of PUFAs marks them for breakdown by phospholipases. The previous are complicated with altered molecular localization to the membrane, impaired membrane barrier function, inactivation or dysfunction of membrane-bound receptors or bound enzymes (*e.g*. ion-motive ATPases), modulation of non-specific permeability for monovalent and divalent ions, water, and high molecular weight compounds and disruption of signalling pathways. Damage of lysosomal membranes facilitates the exit of hydrolytic enzymes, whereas mitochondrial damage membrane releases Ca^{2+}, leading to the activation of Ca^{2+}-dependent enzymes or inducing apoptotic pathways [4, 5, 8, 13-19].

1.2.3. Protein Oxidation

Protein oxidation can occur at the amino group, the carboxyl group, or the side chains that impart the unique properties of amino acids. The side chains of amino acid residues, particularly sulphur containing amino acids (cysteine, methionine) are vulnerable to oxidation. When cysteine residues are oxidized, mixed disulphides between protein thiol groups (–SH), in particular glutathione (GSH) and low molecular weight thiols (S-glutathiolation) are formed. Oxidation of methionine residues generates methionine sulphoxides. Disulphides and methionine sulphoxides are the only oxidized proteins which can be altered back to their native form by disulphide reductases and methionine-sulfoxide reductases respectively.

Other protein oxidation products are nitro-tryptophan, kynurenine, and formylkynurinine from tryptophan; tyrosine-tyrosine cross-linkages, 3,4-dihydroxyphenylalanine, and cross-linked nitrotyrosine from tyrosine; 2,3-dihydroxyphenylalanine, and 2-, 3-, and 4-hydroxyphenylalanine from phenylalanine; glutamic semialdehyde from arginine, α-aminoadipic semialdehyde from lysine; asparagine, 2-oxohistidine, and aspartic acid from histidine; 4- and 5-hydroxyproline pyroglutamic acid, 2-pyrrolidone, and glutamic semialdehyde from proline; 2-amino-3-ketobutyric acid from threonine; hydroxyl residues from leucine and valine.

Oxidative modifications of proteins alter their function and structure or render them active mediators in the progression of inflammatory processes. Oxidatively modified proteins are hydrophobic, due to partial protein unfolding, and susceptible to proteolytic degradation, denaturation, and aggregation with other oxidatively modified biomolecules. During oxidative stress, cells depend primarily on ubiquitin–proteasome-mediated degradation and autophagy for effective removal of damaged or oxidized proteins [4, 5, 8, 13-18, 20, 21].

1.2.4. Advanced Glycation end Products

Advanced glycation end products (AGEs) are very unstable, reactive compounds generated when glucose and other glycating substances, derived from glucose and increased fatty acid oxidation (*e.g*. dicarbonyls such as glyoxal, methylglyoxal and 3-deoxyglucosone) react non-enzymatically with amino groups of proteins, lipids, and nucleic acids. AGEs production can increase beyond normal levels, under certain conditions, such as oxidative stress due to hyperlipidemia or hyperglycemia. AGEs can modify the structure and function of intracellular proteins, whereas certain AGEs activate NAD(P)H oxidase, justifying an innate catalytic oxidative capacity. Plasma proteins modified by AGE precursors bind to RAGE receptors (Receptor for Advanced Glycation End Products) on macrophages, adipocytes, vascular endo-

thelial cells, vascular smooth muscle cells, podocytes, and mesangial cells. Activation of RAGEs damages mitochondrial proteins, perpetuating AGEs' oxidative effects and instigating the generation of intracellular ROS. The latter activate the transcription factor NF-kB, which is redox sensitive and subsequently modulate the expression of a variety of genes, associated with inflammation, including tumour necrosis factor- a (TNF-α), interleukin-6 (IL-6), intracellular adhesion molecule-I (ICAM-I), vascular adhesion cell molecule-I (VCAM-I), monocyte chemotactic protein-I (MCP-I), PAI-I (Plasminogen activator inhibitor-1), implicated in leukocyte activation and chemotaxis [3, 6, 13, 14, 20-27].

1.3. Antioxidant Cellular Defence Mechanisms

Metabolically active and growing cells and high performance tissues, *e.g*. neurons and myocardial cells have high demand for oxygen, engendering massive ROS load, thus are most likely to be markedly affected by ROS burden. Aging, cancer, chronic inflammatory and autoimmune diseases (diabetes, lupus erythematosus, vasculitis, rheumatoid arthritis), cardiovascular diseases (hypertension, atherosclerosis, ischemia/reperfusion injury, obesity), age-related macular degeneration, neurological disorders [Parkinson's disease (PD), Alzheimer's disease (AD), ALS (amyotrophic lateral sclerosis), schizophrenia], fibrotic diseases (pulmonary and liver fibrosis, diabetic nephropathy) and infections (septic shock, hepatitis, HIV) clearly illustrate the impact of oxidative stress on human health [1-3, 5, 28].

In order to preserve a physiological redox balance, cells have a battery of redundant endogenous, antioxidant defences, achieved by enzymatic [catalase, superoxide dismutases (SOD), enzymes of glutathione thioredoxin system, *i.e*. glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST)] and non-enzymatic (metabolic or nutrient -based) pathways. Also, activation of the transcription factor Nrf2/ARE [Nuclear factor (erythroid-derived 2)-like 2/ Antioxidant Responsive Element] protects cells from oxidative stress-induced cell death. Nrf2 is a key regulator of ARE-mediated gene expression of phase II detoxifying enzymes and antioxidant enzymes, *e.g*. NAD(P)H quinone oxidoreductase 1 (NQO1), glutamate-cysteine ligase modifier (GCLM), glutamate-cysteine ligase catalytic (GClc), sulfiredoxin 1 (SRXN1), thioredoxin reductase 1 (TXNRD1), GST, heme oxygenase-1 (HMOX1), multidrug resistance (MDR)-associated proteins and UDP-glucuronosyl-transferase (UGT).

Non-enzymatic antioxidants exert their activity in the absence of enzymatic processes, acting as ROS scavengers or chelating agents. The metabolic antioxidants, *e.g*. GSH, are synthesized from the amino acids L-cysteine, L-glutamic acid, and glycine, lipoic acid, L-arginine, urate, transferrin, melatonin (N-acetyl-5- methoxytryptamine), ubiquinol (co-enzyme Q) are formed during cellular metabolic reactions, whereas nutrient antioxidants are acquired from dietary sources, *e.g*. α-tocopherol, vitamin C, carotenoids, flavonoids, anthocyanins, cysteine, fatty acids, and polyphenols [1, 2, 4-6, 8, 21, 28, 29].

Considering the central role of oxidative stress in cellular damage, manipulation of ROS cellular content may represent a promising treatment option to prevent or alleviate symptoms of age-associated degenerative diseases. In this regard, carotenoids and retinoids have attracted wide attention as promising sources of pharmaceuticals with low toxicity for the prevention and treatment of a broad spectrum of diseases, due to their antioxidant, anti-inflammatory, and immunomodulatory effects [30, 31]. The most abundant carotenoids in human serum are β-carotene (vitamin A precursors), α-carotene, lycopene, lutein, β- cryptoxanthin, zeaxanthin and astaxanthin. Indeed, vitamin A can be obtained from preformed vitamin A sources, such as retinol and retinyl esters or from provitamin A carotenoids (α-carotene, β-carotene, β-cryptoxanthin), which convert to the active retinol in the body. Nevertheless, their toxicity and storage in adipose tissue remain important limiting factors for their usage at high therapeutic doses. On the other hand, carotenoids contained in saffron are unique in comparison to most other carotenoids because of their water solubility, attributed to sugar moieties bound to the carboxylic acid groups appended on crocetin. Hence, they can spread easily throughout the body, reaching every tissue, whereas the excess is excreted and not stored in tissues, such as adipose tissue or liver. Indeed, saffron is regarded as a valuable plant source for drug development, endowed with pleiotropic health-promoting effects and a more favourable safety profile. In this review, we focused on the capacity of saffron extracts and its components, to attenuate oxidative stress, providing a potential means in preventing or decreasing cognitive deterioration in Alzheimer's disease and motor impairment in Parkinson's disease [2-6, 28, 32, 33].

2. CHEMISTRY OF *Crocus Sativus* L

Among medicinal plants there is growing interest in *Crocus Sativus* L., known for its various pharmacological properties for over 3,600 years ago. It is a perennial, stemless herb, belonging to *Iridaceae* family, the line of *Liliaceae*, cultivated in various countries such as Greece, Italy, Spain, Israel, Morocco, Turkey, Iran, India, China, Egypt and Mexico [34, 35].

Saffron, the golden spice, is the flower's dried stigma and is known for its medicinal properties, as it possesses anticancer [35], genoprotective, antioxidant, anti-inflammatory [35, 36], antidiabetic [35], anti-atherosclerotic [37, 38], hypolipidaemic [38, 39], hypotensive [36, 40-42], hepatoprotective [25, 39], antidepressant [43-47], anticonvulsant [46], anxiolytic and hypnotic effects [36, 38, 46, 48-55], it improves learning [56, 57], cognitive performance [36, 48-55] and memory impairment [36, 48-55], even in AD [38].

The plant is characterized by its three long red stigmas, joined by the style, three yellow stamens and six purple tepals. Saffron, has distinctive colour, flavour and smell, containing more than 150 volatile and non-volatile compounds. The volatiles consist of more than 34 constituents, mainly terpene alcohols, terpenes, and their esters, whereas non-volatiles include crocins, carotenes, crocetin and picrocrocin [34, 58].

Saffron has three principal active substances (i) crocins, which are unusual water-soluble carotenoids (glycosyl esters of crocetin) and give the red pigmentation of stigmas, (ii)

Table 2. Crocetin and crocin derivatives of saffron [94, 114].

Compound	Sugar Moieties	Chemical Formula	Isomer Occurrence in Saffron
Crocin-1	R_1 = *β*-D-glucosyl R_2 = H	$C_{26}H_{34}O_9$	trans
Crocin-2	R_1 = *β*-D-gentiobiosyl R_2 = H	$C_{32}H_{44}O_{14}$	cis–trans
Crocin-3	$R_1 = R_2$ = *β*-D-glucosyl	$C_{32}H_{44}O_{14}$	cis–trans
Crocin-4	R_1 = *β*-D-gentiobiosyl R_2 = *β*-D-glucosyl	$C_{38}H_{54}O_{19}$	cis–trans
Crocin-5	$R_1 = R_2$ = *β*-D-gentiobiosyl	$C_{44}H_{64}O_{24}$	cis–trans
Crocin-6	R_1 = 3 *β*-D-glucosyl R_2 = *β*-D-gentiobiosyl	$C_{50}H_{24}O_{29}$	cis–trans
Dimethylocrocetin	$R_1 = R_2 = CH_3$	$C_{22}H_{28}O_4$	cis –trans
Crocetin	$R_1 = R_2$ = OH	$C_{20}H_{24}O_4$	cis –trans

picrocrocin, a monoterpene glycoside, mainly responsible for its distinct bitter flavour, and (iii) safranal, the main component of the essential oil that gives the characteristic odour to saffron. Picrocrocin, (4-(*β*-D-gluco-pyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde; $C_{16}H_{26}O_7$), is a monoterpene glycoside of the aglycone 4-hydroxy-2,6,6-trimethyl-1-carboxaldehyde-1-cyclohexene (HTCC). It is a degradation product of the zeaxanthin carotenoid and a precursor of safranal. Picrocrocin can be converted to safranal during the drying process, in the presence of glycosidases or elevated temperatures. Safranal, (2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde; $C_{10}H_{14}O$), is a monoterpene aldehyde, which is formed directly by natural deglycosylation of picrocrocin or *via* HTCC. It is the most abundant (30-70%) of total volatiles and comprises 0.001–0.006 % of dry matter of saffron.

The crocins family are hydrophilic carotenoids that are either mono- or diglycosyl polyene esters of a dicarboxylic acid, crocetin (2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptaenedioic acid; $C_{20}H_{24}O_4$), in which D-glucose and/or D-gentiobiose occur as carbohydrate residues. In saffron all crocin derivatives, except crocin-1, occur as pairs of cis-trans isomers, among which trans-crocins -4 and -5 are the most abundant. Crocin derivatives in saffron are trans-crocetin (*β*-D-gentiobiosyl) ester, trans-crocetin di-(*β*-D-glucosyl) ester, trans-crocetin (*β*-D-glucosyl)-(*β*-D-gentiobiosyl)bester, cis-crocetin (*β*-D-glucosyl)-(*β*-D-gentiobiosyl) ester, trans-crocetin di-(*β*-D-gentiobiosyl) ester ($C_{44}H_{64}O_{24}$; the most abundant component of crocins) and cis-crocetin di-(*β*-D-gentiobiosyl) ester. The presence of sugar moieties attached to the terminal -COOH groups of the crocetin backbone plays a role in cell membrane penetration. Crocetin (8,8-diapo-8,8'-carotenoic acid) (CRT) is an amphiphilic natural carotenoid with high solubility in organic solvents (Table **2** and Fig. **1**) [58-63].

Other constituents include carotenoids (lycopene, alpha-, beta-, gamma-carotene, zeaxanthin, phytoene, phytofluene, mangicrocin and xanthone-carotenoid glycosidic conjugate), phenolic and flavonoid compounds. Acceptable quality saffron contains roughly 30% crocins, 5–15% picrocrocin, and 2.5% volatile compounds, including safranal [29, 32, 35, 58-68].

3. ANTIOXIDANT CAPACITY OF SAFFRON, AND ITS MAIN CONSTITUENTS

Saffron methanol extract solutions at a concentration above 2000 ppm displayed high antioxidant activity of about 40-50%, as assessed by DPPH (1,1-diphenyl-2- picryl-hydrazyl; $C_{18}H_{12}N_5O_6$) free radical scavenging activity. Methanolic saffron extracts possessed higher antioxidant properties, compared with carrots and tomatoes at high concentrations (600 and 300 μg/mL), but their antioxidant activ-

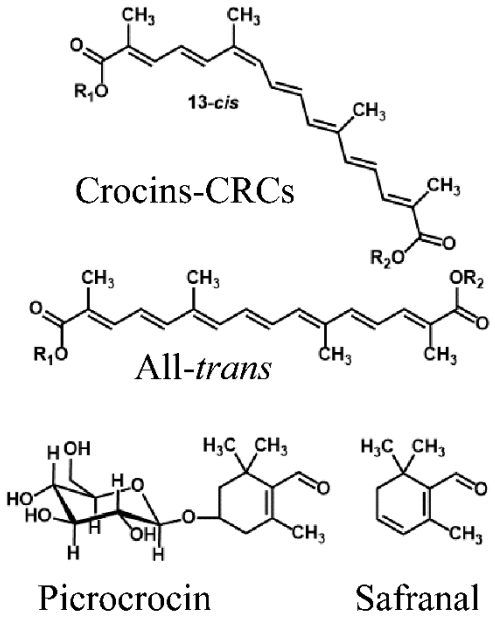

Fig. (1). Structures of saffron components.

ity was lower than that of known antioxidants, such as BHT and α- tocopherol. However, the reaction of α- tocopherol was essentially completed after 1 minute, whereas the antioxidant activity of vegetables and saffron extracts were increasing, within a period of 20 minutes [69].

Saffron's antioxidant potential and free radical scavenging activities could be attributed to its phenolic and flavonoid content, with gallic acid and pyrogallol identified as the main phenolic and flavonoid compounds, respectively. Phenolics and flavonoids are important precursors of most types of oxidizing molecules [32, 70]. DPPH free radical activity and FRAP (Ferric Reducing Activity of Plasma) assays revealed that free radical activity of methanol-bound saffron stigma extract was stronger compared to boiling water extract, followed by the ethanol-bound stigma extract, due to differences in total phenolics and flavonoids. Antioxidant activity increased in a dose-dependent mode.

Scavenging activity of free radicals of saffron extracts and antioxidant standards at a concentration of 300 μg/mL were in ascending order α- tocopherol > BHT (butylated hydroxytoluene) > methanol > boiling water > ethanol. The IC50 of α- tocopherol, BHT, and methanol, boiling water and ethanol extract were calculated 89.77, 60.39, 210.79, 255.44, and 299.44 μg/mL, respectively [69, 71]. Moreover, comparison between ethanolic and water extracts of saffron revealed that the ethanolic extract had the highest gallic acid content (67.62 mg/g), as verified by DPPH and FRAP assays in which ethanolic extract exerted the strongest antioxidant capacity [72].

The main bioactive constituents of saffron crocins and safranal also reduce the overload of oxidative stress. In fact, crocins exhibited profound concentration-dependent antioxidant capacity, as at concentrations of 500 and 1000 ppm DPPH radical scavenging activity was calculated 48% and 64%, respectively, whereas safranal's at concentrations of 500 ppm was found 34% [64].

In another research CRT and to a lesser extent dimethylocrocetin (DMCRT) also exhibited significant antioxidant properties, equal to well-known antioxidants BHT and Trolox (hydrophilic vitamin E). IC50 values of CRT, DMCRT, BHT and Trolox were calculated 17.8, 40, 5.2 and 5.3 μg/mL. The results were in accordance with another study, in which IC50 values of CRT, DMCRT and safranal were calculated 18 ± 1 μg/mL, 40 μg /mL and 95 ± 1 μg /mL, respectively [73]. The antioxidant activity of CRT is dose-dependent, whereas of DMCRT, it reaches a certain point and then it starts to diminish. The superior scavenging activity of CRT, as compared with DMCRT could be attributed to their structural differences. Although they have the same length of conjugated double bonds, they differ in the presence of a hydroxyl moiety in the carboxylic group in CRT, which reacts more easily with free radicals. On the contrary, DMCRT has a methyl ester group at the end of the unsaturated hydrocarbon chain, rendering DMCRT a less effective antioxidant [73, 74]. Also, the presence of sugar groups, attached to the terminal -COOH of CRT backbone allows extensive distribution and penetration of CRT through lipid bilayers of cell membranes [75].

4. ROLE OF OXIDATIVE STRESS IN NEURODEGENERATION

The brain, is a relatively small organ by mass, yet it requires about 20% of body's total oxygen due to its high metabolic activity. Neurons produce large quantities of ROS, during the conduction of electrical potential across neuronal cell membranes. The main sources of ROS in the CNS are the mitochondrial respiratory chain, the xanthine oxidases, the NADPH oxidases, the lipooxygenases, COX, and via non-enzymatic processes, like dopamine and nor-adrenaline auto-oxidation. In CNS transient production of ROS is implicated in signalling at synapses. ROS at hippocampal excitatory synapses (CA1 area, dentate gyrus) act as messengers during NMDAR (N-methyl-D- aspartate receptor)-dependent long term potentiation (LTP), which refers to persistent strengthening of synapses based on recent patterns of activity (activity-dependent synaptic plasticity), constituting the cellular basis of memory and learning [56].

Peroxisomes in oligodendrocytes engender massive concentrations of ROS, as they are found in large numbers during active myelination. When aberrant and prolonged glial activation surpasses the protective threshold, especially during neuroinflammation or imbalanced redox cycling, glial cells induce iNOS expression, release cytokines, such as TNF-α, IL-1, and IL-6, and chemokines and generate more ROS, hence neuroinflammation could be recognized both as a cause and a consequence of CNS oxidative stress. Astrocytes and oligodendrocytes contain an inducible lipocalin, ApoD (apolipoprotein D), which is secreted during oxidative stress and neurodegeneration, protecting cells from oxidation and apoptosis, *via* up-regulation of the cellular antioxidant pool. However, excessive •NO production may inhibit ApoD expression, thus glial reactivity may represent a critical switch between neuroprotection and neurodegeneration.

Abnormally elevated ROS levels have deleterious effects in CNS, as neuronal cells are post-mitotic cells, thus are incapable of proliferation and therefore, are particularly vulnerable to oxidative stress, leading to irreversible neuronal damage. Also, the brain is rich in PUFAS which are labile to peroxidation and oxidative modification. MDA-mediated covalent alteration of proteins, especially of albumin initiates immune responses toward this protein, aggravating the course of neuroinflammation. Generation of 4-HNE *via* lipid peroxidation interferes with the function of ion-motive AT-Pases, glucose and glutamate transporters. Advanced oxidation protein products and AGEs are considered exceptionally pathogenic, as they engender ROS, independently promoting further spreading of oxidative stress and neuroinflammation, acting as mediators in the production of various cytokines and adhesion molecules. Interestingly, cells in CNS, despite the relatively high energy turnover, have a relatively poor battery of antioxidant systems, *e.g.* about 10% of the antioxidant activity of hepatocytes.

4.1. Role of Oxidative Stress Induced-mitochondrial Dysfunction in Neurodegeneration

Neuronal cells depend heavily on aerobic respiration for ATP production and have a relatively limited potential for

anaerobic metabolism, hence are rich in mitochondria to meet their high energy demands. However the drawback is the massive production of ROS, as normal by-products during oxidative phosphorylation, which may overwhelm the cellular antioxidant mechanisms. With advancing age and during neuroinflammation and/or neurodegeneration, neurons exhibit impaired mitochondrial function, leading to reduced production of ATP and NAD^+ (*Nicotinamide Adenine Dinucleotide),* in parallel with and increased ROS generation, due to alterations in expression and inadequate efficiency of the complexes of the respiratory chain. It is well-established that damaged mitochondria produce massive amounts of ROS. Also, neuronal mitochondria are less equipped to adapt to oxidative stress, undergoing sluggish turnover [76].

During uncontrolled nitroxidative stress, mitochondrial proteins undergo post-translational modifications, such as oxidation, nitration, acetylation, carbonylation, palmitoylation, phosphorylation, glycosylation, S-nitrosylation, myristoylation and protein adduct formation. Modified proteins are inactivated, contributing to compromised mitochondrial function, ATP depletion and initiation of cellular death or apoptosis. Furthermore, lipids and mtDNA are themselves directly susceptible to effects of ROS exposure.

Complex I is extremely sensitive to oxidative stress since it is the first to be exposed to oxidative modifications, it contains iron–sulphur clusters, which can be a site of direct ROS attack and seven subunits of its catalytic core are encoded by mtDNA. Acute cellular exposure to ROS also inactivates iron-sulphur (Fe-S) centres of electron-transport chain complexes I, II, and III (*e.g*., succinate dehydrogenase/SDH and NAD dehydrogenase), and tricarboxylic acid cycle aconitase, impairing energy production at mitochondria.

mtDNA is a critical target for initiation, amplification and propagation of cellular oxidative damage, as it is particularly vulnerable to ROS-mediated mtDNA mutations. Once mtDNA is damaged, the expression and function of proteins essential for electron transport are decreased, resulting in increased electron reduction of O_2 to form $O_2\bullet^-$ and initiation of a cycle of oxidative chain reactions and organelle dysregulation, eventually triggering apoptosis.

The inner mitochondrial membrane is permeable only to neutral small molecules, thus it is challenging for exogenous antioxidants to reach mitochondria. Nonetheless, the inner mitochondrial membrane is prone to lipid peroxidation, as it is in close proximity to the site of ROS production. Peroxidation of mitochondrial phospholipids increases the permeability of the inner mitochondrial membrane to protons, alters its fluidity and impairs functions of various transporters and enzymes of the respiratory chain in inner and outer membranes. Also, ROS increase mitochondrial permeability by oxidation of thiol groups on the adenine nucleotide translocator, which is part of the mitochondrial permeability transition pore (mPTP). The mPTP is a non-specific pore with large conductance, formed by proteins residing in the inner and outer mitochondrial membrane, *e.g*. TSPO (translocator protein)/peripheral benzodiazepine receptor and cyclophilin D, although little it is known about its structure. The pore is induced by mitochondrial Ca^{2+} overload, oxidative stress, ATP depletion, receptor-mediated death signals (Fas and TNF-R), and aberrant expression of pro-apoptotic and anti-apoptotic Bcl-2 (B-cell lymphoma 2) family proteins. When activated the pore augments the permeability of inner mitochondrial membrane to solutes with molecular mass of up to 1.5 kDa, leading to mitochondrial osmotic swelling, dissipation of the inner membrane potential (depolarization), rupture of the outer mitochondrial membrane, uncoupling of oxidative phosphorylation, increased $^{\bullet}O{-}_2$ production, outflow of matrix Ca^{2+} *via* Na^+/Ca^{2+} or H^+/Ca^{2+} exchangers and nonspecific leakage of intermembrane space proteins to the cytosol, *e.g*. cytochrome c, AIF proteins (Apoptosis inducing factor), Smac/DIABLO, endonuclease G, and procaspases, followed by apoptotic effector caspases activation.

ROS are also implicated in neuronal cell death via elevating Bax expression and the Bax/Bcl-2 ratio. Bcl-2 resides in the outer mitochondrial membrane, preventing the release of cytochrome c. Also, Bcl-2 regulates the activity of mPTP, thus inhibiting Ca^{2+} overload and attenuating apoptosis. Bax is inactive until it is translocated to mitochondria to bind Bcl-2, provoking cytochrome c release into the cytosol. Cytochrome c binds apoptotic protease activating factor 1 (Apaf- 1) and actuates caspases to trigger apoptosis. It regulates the activation of caspase-9 which further activates caspase-3. The latter activates calpains, caspases -activated endonuclease CAD/DFF40 and promotes the cleavage of DNA repair enzyme PARP [Poly (ADP-ribose) polymerase]. The previous mediate the induction of stress-induced neuronal cell apoptosis [18, 26, 76-78].

4.2. Role of Oxidative Stress Induced-alterations in Ca^{2+} Homeostasis in Neurodegeneration

Ca^{2+} has a major role in intracellular signalling, implicated in the translation of extracellular stimuli into intracellular processes, such as gene expression, neurotransmission, cell proliferation, and apoptosis. It is also implicated in activation of several mitochondrial enzymes, *e.g*. 2-oxoglutarate dehydrogenase, pyruvate dehydrogenase, and NAD-linked isocitrate dehydrogenase. The influx of Ca^{2+} in neurons is mediated *via* voltage-dependent (L-type Cav 1.3 calcium channels) and ligand-gated channels (*e.g*. NMDARs) located on the plasma membrane. The influx of Ca^{2+} in neurons is critical for the release of neurotransmitters from presynaptic terminals and for responses of the postsynaptic neuron. Intracellular Ca^{2+} homeostasis is preserved through a plasma membrane $Na+/Ca^{2+}$exchanger, plasma membrane and ER Ca^{2+}-adenosine triphosphate synthases, Ca^{2+}-binding proteins such as calbindin and parvalbumin, mitochondrial and ER sequestration. Excessive ROS generation alters Ca^{2+} homeostasis in mitochondria by oxidation of specific thiol groups in proteins and the cellular energy status *via* inactivation of energy-dependent calcium-ATPases, thus triggering Ca^{2+} release from mitochondria and intraneuronal Ca^{2+} -overload.

When Ca^{2+} influx and efflux from intracellular pools during oxidative stress exceeds the capacity of calcium regulatory mechanisms calcium-dependent enzymes are activated, like phosphatases and proteases, damaging directly cell structures, such as NOS, COX, phospholipase A2, and calpain 1. Also, Ca^{2+} sustained mitochondrial overload induces

mPTP, activates neuronal cell death pathways and $^{\bullet}O^{-}_{2}$ burst by activating oxygenase enzymes of the electron transport chain or changing MTP (mitochondrial transmembrane potential) and increasing electron leakage from mitochondria, which further deranges Ca^{2+} homeostasis, thus instigating a damaging cycle, resulting to neurodegeneration [18, 26, 77, 78].

4.3. Role of Oxidative Stress Induced-NMDARs Overstimulation in Neurodegeneration

Glutamate is the most prominent excitatory neurotransmitter in mammalian CNS, involved in learning, memory and neuronal plasticity. It acts through both ionotropic receptors (iGluRs), e.g. N-methyl-D- aspartate (NMDA), kainate and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate (AMPA) receptors and metabotropic receptors (mGluRs), e.g. mGluRs 1-8. Overstimulation of NMDARs after abnormal release of excitatory neurotransmitters, and reduced uptake and metabolism of glutamate by glial cells is an additional mechanism implicated in oxidative stress-induced neurodegeneration. ROS are implicated in up-regulation of functional NMDARs subunits NR1, NR2B, NR2C, and NR2D, excessive stimulation and PKC-dependent sensitization of neuronal and cerebrovascular endothelium NMDARs, all implicated in enhanced glutamate excitotoxicity. NMDARs pronounced activation is associated with abnormal and sustained release of stored synaptic glutamate, leading to massive Ca^{2+} influx and Ca^{2+}-induced mitochondrial dysfunction. The latter are all complicated with ATP depletion, inhibition of the Na^{+}-K^{+} ATPase profound loss of ionic gradients, membrane depolarisation and finally neuronal cell death [18, 26, 77, 78].

Activation of neuronal NMDARs can also induce $\bullet O_2^-$-mediated oxidative stress in neighbouring neurons and astrocytes. NMDAR-generated $O_2^-\bullet$ originates from mitochondria and via activation of a NADPH oxidase isoform NOX2, both pathways activated as a result of alterations in Ca^{2+} homeostasis. NOX2 releases $O_2^-\bullet$ to the extracellular space which can react with NO, generated by neurons in response to NMDARs activation, to yield peroxynitrite and other species that readily react with cell constituents, thus propagating excitotoxic injury. ROS generation and depletion of cellular energy stores can lead to additional release of glutamate and retrograde up-regulation of NMDARs, representing a pathological feedback mechanism. Moreover, ROS-triggered up-regulation and sensitization to glutamatergic stimuli of NMDARs and excessive ROS production generated in end-feet and endothelia are instrumental to blood-brain barrier disruption and down-regulation of tight junctions, a common feature of neurological diseases, such as AD and PD [79-82].

4.4. Role of Oxidative Stress Induced-proteasome and Autophagy Impairment in Neurodegeneration

A common feature in neurodegenerative conditions is formation and aggregation in the extracellular space of amyloid fibrils from a range of soluble proteins or their proteolytic fragments. Occasionally, fibrillary protein deposits are located inside neurons or glial cells. The deposition of dysfunctional and insoluble proteins, as verified by ubiquitin-immunopositive inclusion bodies, is probably linked to a decline or impairment of proteasome activity and autophagy/lysosomal proteolysis. During oxidative stress, proteasome subunits or ubiquitin-conjugating enzymes are susceptible to oxidative modifications, such as glutathionylation, carbonylation, S-nitrosylation, and HNE modifications, leading to oxidative stress-induced impairment of protein degradation by proteasomes and accumulation of ubiquitinated proteins.

Autophagy is a significant process in lysosomal degradation, regulating cellular metabolism and homeostasis. This takes place by elimination of intracellular aggregated proteins and damaged organelles within lysosomal vesicles. During oxidative stress, autophagy is dysfunctional and abnormal accumulation of autophagosomes has been found in dystrophic neurites. Deficiencies in autophagy flux, autophagosome creation or clearance of autophagic vesicles can lead to neuronal cell death. Given the critical role of autophagy in regulating turnover of defective mitochondria, it is not surprising that inhibition of autophagy is directly linked to disproportionate ROS generation within the CNS [83].

4.5. Role of Oxidative Stress Induced-inflammation in Neurodegeneration

Oxidative stress can initiate inflammatory cascades, e.g. generation of pro-inflammatory cytokines [IL-1b, IL-6, IL-8, TNF-α, TGF-β (Transforming growth factor beta)] and expression of adhesion molecules (integrins, selectins, immunoglobulin superfamily molecules), via an interplay between leukocytes (granulocytes, monocyte/macrophages and lymphocytes), astrocytes, microglial, and endothelial cells. Also, it can up-regulate the activity of phospholipase A2, LOX and COX, generating intermediates of free fatty acid (FFA) metabolism, like arachidonic acid, thromboxane and leukotrienes, which favour cerebral oedema, vasoconstriction, and platelet aggregation [18, 79, 84, 85].

Oxidative stress plays a critical role in the pathogenesis of neuro-inflammation and neurodegeneration, offering the possibility for adopting anti-oxidative treatments early in the course of neurodegeneration, thus being able to reverse or delay later phases or achieve remission.

5. ANTIOXIDANT ACTIVITY OF SAFFRON, AND ITS MAIN CONSTITUENTS, IN TERMS OF PREVENTION OR THERAPY OF NEURODEGENERATIVE DISEASES

5.1. Alzheimer's Disease

5.1.1. Oxidative Stress in Alzheimer's Disease

Alzheimer's disease is the most common cause of dementia among people over 65 years old and accounts for 60-80 % of dementia cases. The hallmark of AD is misprocessing of amyloid precursor protein (APP) by β- and γ-secretases, leading to overproduction of toxic amyloid β (Aβ) monomers, which eventually oligomerize to form pentamer/hexamer units (paranuclei). Paranuclei are the smallest structures that can be oligomerized into larger intracellular forms, such as large oligomers, protofibrils and insoluble fibrils. Monomers, paranuclei and large oligomers are predominately unstructured with small amounts of β-sheet/β-turn and α-helix, whereas during the formation of protofibrils, spe-

cific conformational changes are observed, causing the structures of α-helix and β stranded structures to change into β-sheet/β-turn structures. A*β* fragments accumulate in the extracellular space until an initial nucleation event occurs, leading to aggregation and deposition of *β*-amyloid dense senile plaques. Intracellular fibrillary oligomeric isoforms of A*β* peptides, formed earlier in the course of AD are thought to exert neurotoxicity and not the extracellular amyloid plaques [86].

Hyperphosphorylation of microtubule-associated protein tau, which accumulates to intracellular tau neurofibrillary tangles (NFT) is also implicated in the pathophysiology of the disease. Tau is normally a highly soluble microtubule-associated phosphoprotein, whose primary intracellular role is stabilization of microtubules by forming assemblies of tubulin subunits, preserving cell morphology, and aiding axonal transport along the microtubule network. Its degree of phosphorylation regulates microtubule polymerizing activity. In the normal, adult, human brain tau contains 2-3 moles phosphate per mole of tau, whereas in AD tau is three- to four-fold hyperphosphorylated as compared to the brain of normal adults. Hyperphosphorylation of the protein depresses its biological activity, and tau dissociates from microtubules, leading to abnormal accumulation of hyperphosphorylated tau proteins. Also, Tau hyperphosphorylation is correlated with mitochondrial morphology defects and A*β*-mediated neuronal death. Tau fibrillogenesis proceeds *via* formation of oligomeric nucleating cores during an initial lag-phase, followed by a period of rapid fibril growth, propelled by tau hyper-phosphorylation. The fibrillary tau deposits occupy a cross-*β*-fibril structure. NFT formation leads ultimately to neuronal dysfunction, disintegration of transport system in axons and cell death.

The most common isoforms are $A\beta_{40}$ and $A\beta_{42}$; the shorter form is the most abundant, but the latter is the more fibrillogenic and neurotoxic, as it possesses two extra hydrophobic amino acids [87, 88]. $A\beta_{42}$ integrates as a small, soluble aggregate within the membrane lipid bilayers, in a-helical conformation, due to its increased hydrophobicity, initiating cellular damage, such as lipid peroxidation, and protein oxidation. The residue 35 of A*β*-peptide is a methionine an easily oxidized amino acid, which appears to be important for A*β* -associated toxicity and oxidative stress initiation. Methionine residues shield proteins against oxidation, as promoters of alpha-helix secondary structures, or as oxidation-sensitive sites which regulate the redox state of cells and the action of certain repair or chaperone enzymes. Alkylsulphides react with oxygen in a metal-independent manner, producing a sulfoxide, in our case methionine sulfoxide (MetSOx). Methionine sulfoxide reductase (MSR) converts MetSOx back to methionine, suggesting that MSR has a possible antioxidant role. Interestingly, in AD the activity of MSR is low, thus further oxidation of MetSOx can yield a sulfone product, in an unfavourable and irreversible reaction. Methionine in $A\beta_{42}$ –inserted in the lipid bilayers generates a methionine sulfuranyl radical (MetS•) by a one-electron oxidation. The intermediate radical abstracts a hydrogen atom from a neighbouring unsaturated lipid, forming a carbon-centred L•, which in turn reacts with molecular oxygen to produce LOO•. Hydrogen abstraction from neighbouring lipids engenders lipid hydroperoxides (LOOH) and other L•, thus potentiating ROS chain reactions. MetS• reacts with other moieties of methionine to form an a(alkylthio)alkyl radical of methionine (-CH2-CH2-S-CH2 or -CH2-CH-S-CH3), providing potential substrates for oxidation, leading to the formation of peroxyl radicals, thus propagating ROS generation [4, 5, 8, 13-18, 89-91].

Lipid peroxidation of PUFAs in neuronal cell membranes and its by-products (e.g., HNE, acrolein) are markedly elevated in neurodegenerative diseases, inflicting cellular damage. HNE and acrolein levels are increased in the brain of patients with early AD. HNE-modified *β*-amyloid selectively inhibits the 20S proteasome, linking proteasome impairment to A*β*-induced oxidative stress in AD. Also, HNE produced by $A\beta_{42}$ binds to the glutamate transporter, EAAT2 (excitatory amino acid transporter 2) in synaptosome. The receptor is responsible for glutamate sequestration by astroglia (astrocytes, microglia, oligodendrocytes), in which it is converted to glutamine by glutamine synthetase, an oxidation-prone enzyme. A*β*-induced oxidative inhibition of glutamate transporters results in significant accumulation of extracellular glutamate and sustained activation of neuronal excitatory NMDARs. Also, A*β* peptides and ROS inhibit the activity of glutamine synthetase via protein oxidative modifications [21, 27, 33, 90, 92-95].

The transition metals, Zn^{2+}, Cu^{2+}, and Fe^{3+}, are altered in amyloid plaques and the brain of subjects with AD. A*β* possesses three histidine residues at positions 6, 13, and 14. Histidine is a strong metal ion coordinator in proteins and peptides. However, even small alterations in the coordination may have an effect on protein aggregation. Histidine residues catalyse the reduction of abundant in A*β* peptide-bound Cu^{2+} and Fe^{3+}, generating H_2O_2 (SOD-like properties). The latter converts to •OH, in the presence of Cu^{+} and Fe^{2+} [76, 91, 94, 96-98].

Extracellular A*β* monomers and oligomers enter intracellular environment via clathrin-mediated endocytosis, NMDARs and acetylcholine receptors. Intracellular A*β* can be embedded in mitochondrial inner membrane or matrix, as they harbour a chimeric targeting signal comprised of a N-terminal hydrophobic ER signal peptide, followed by a cryptic mitochondrial targeting signal, whereas a more C-terminally located domain acts as a mitochondrial translocation arrest sequence. Residues at 40, 44, and 51 of A*β* which harbor a positive charge are critical components of the mitochondrial targeting signal. A*β* peptides form stable translocation intermediate complexes with translocase of the outer membrane and also link translocase of the outer membrane with translocase of the inner membrane together. There is evidence that mitochondrial A*β* accumulation precedes the formation of extracellular plaques. A*β* disturbs the activity of mitochondrial electron transport chain complexes - specifically complexes III and IV (cytochrome c oxidase), whereas Tau deregulates complex I, all leading to impaired mitochondrial function, suppressed ATP generation and increased mitochondrial ROS generation. A*β* oligopeptides can also directly initiate ROS formation via activation of the membrane-bound NADPH oxidase. ROS result in loss of MTP and alteration in mitochondrial membrane ultrastructure and permeability, thus leading to a further burst in ROS

generation, mPTP opening and release of cytochrome c [99-102]. Also, mitochondrial Aβ may interact with a peptidyl-prolyl isomerase F of mitochondrial matrix, cyclophilin D (CypD). CypD potentiates ROS generation, causes synaptic dysfunction, and promotes mPTP opening [102].

In AD, ROS are associated with formation of di-tyrosine cross-linking between Aβ oligomers, leading to enhanced oligomerization and aggregation of misfolded Aβ peptides, thus self-propagating oxidative damage. Aggregation of misfolded or unfolded proteins, often results in activation of multistep pathways to eliminate the unwanted proteins, a homeostatic mechanism termed ER stress, which is accompanied by translational repression, activation of innate immune pathways or cellular death. Endoplasmic reticulum stress is linked to neuroinflammation *via* intersecting signalling pathways, *e.g*. ROS generation and pro-inflammatory cytokines. Besides, protein folding is an energy demanding process, occurring in oxidizing conditions and can be compromised if cells are depleted of ATP, due to mitochondrial dysfunction [103].

Aβ_{42} mediates influx of Ca^{2+} into the neuron, deregulating intracellular Ca^{2+} homeostasis, which further accelerates Aβ aggregation, facilitates the formation of early protofibrillar structures and fibrils, in addition to mitochondrial dysfunction. Aβ can also induce ER stress, further disrupting Ca^{2+} homeostasis. A_{1-40} and A_{25-35} in the presence of Ca^{2+} decrease MTP and the capacity of brain mitochondria to sequester Ca^{2+} and induce opening of mPTP, release of apoptogenic factors and activation of caspase-dependent intrinsic apoptosis pathway. Extracellular Aβ can activate extrinsic apoptosis via activation of cell surface death receptors (FAS-receptor) [99, 104].

An additional target of Aβ in mitochondria is a zinc-metalloendopeptidase, hPreP (human Presequence Protease), implicated in mitochondrial degradation of Aβ_{40} and Aβ_{42}, cleaving the hydrophobic C-terminal Aβ_{29-42} segment which is prone to aggregation. The enzyme has two highly conserved cysteine residues (Cys527 and Cys90) in close proximity to each other, which under oxidizing conditions form a disulphide bridge locking hPreP in a closed conformation and hindering binding of substrates. Thus, under conditions of Aβ -mediated ROS production in mitochondria, the mitochondrial degradation and clearance of Aβ is impaired, due to hPreP inactivation. Proteasome and autophagy-mediated proteolysis are also impaired, leading to abnormal intracellular accumulation of neurotoxic Aβ and secretion of Aβ to extracellular space. Autophagic vacuoles are sites of intracellular Aβ production as accumulated vacuoles in neuronal cells are rich in APP content and active enzymes for Aβ production [83, 101, 102].

Also, Aβ binds to a mitochondrial matrix protein A-binding alcohol dehydrogenase (ABAD), a member of the short-chain dehydrogenase/reductase family which catalyses the reduction of aldehydes and ketones with NAD+ /NADH as its cofactor and oxidation of alcohols for energy production. Decreased ATP generation could also be related with depressed consumption of glucose in the brain, a very early sign of AD, detectable up to three decades prior to the expected onset of clinical symptoms of early onset disease. Aβ-binding impairs ABAD function, reducing in an addition way the activity of respiratory enzymes, hence inducing ROS formation and causing ATP depletion. ABAD is important for mitochondrial ketone utilization, as it promotes generation of acetyl-CoA to enter Krebs cycle*,* an effect of particular importance in stress situations [102]. Also, oxidative modifications of enzymes important in cellular energy production, such as ATP-synthase, a- enolase, and lactate dehydrogenase (LDH) have been found in brains of subjects with AD. The previous are complicated with a significant pro-apoptotic shift, as indicated in alterations on Bax and Bcl-2 expression. In addition, a-enolase possesses non- glycolytic properties, important to cell survival (neurotrophic factor), hypoxic stress regulation and in Aβ clearance. [90, 101, 105-107].

Aβ amyloid peptides can activate the sphingomyelin pathway, via phosphorylation of magnesium-dependent neutral sphingomyelinase (nSMase) by c-Jun N-terminal kinase (JNK) and a rapid increase in cellular ceramide levels. Hydrolysis of sphingomyelin generates ceramide as a second messenger, which modulates cell apoptosis via several pathways, such as inhibition of pro-survival kinases, e.g., PKC (Protein kinase C), and Akt/PKB (Protein kinase B), and activation of stress-activated kinases, e.g., PKC, JNK, kinase suppressors of Ras (KSR), and protein phosphatases, e.g., protein phosphatase 2A (PP2A) and protein phosphatase 1 (PP1). Ceramide also, affects MTP, as it forms channels or targets MTP-controlling proteins, such as Bcl-2, leading to the translocation to cytoplasm of cytochrome c and AIF proteins, followed by caspase-3 activation. The previous mediate the induction of stress-induced neuronal cell death [108, 109].

Aβ- induced stress stimuli modifies cellular signalling pathways, initiating tau hyperphosphorylation, at T231 residue through activation of p38 MAPKs (mitogen-activated protein kinases), a major oxidative stress sensor. MAPKs are activated via oxidative modifications and inactivation of their corresponding MAPKs phosphatases by inactivating redox-sensitive critical cysteine residues. MAPKs phosphatases dephosphorylate activated upstream kinases of each MAPKs. The activated p-p38K phosphorylates many target proteins, including any of 80 serine and threonine residues and 5 tyrosine residues of the microtubule-associated Tau proteins. A significant increase in the content and activation of p-p38K and its upstream activator MAPKK6 (mitogen-activated protein kinase 6), has been detected in early AD [76, 110, 111].

Oxidation and down-regulation or inactivation by ROS of Pin1 (peptidyl-prolyl cis-trans isomerase NIMA-interacting 1) in neurons has gained much interest in AD. The enzyme is implicated in regulation of neuronal cell cycle checkpoints and phosphorylation status of cellular molecules. It modulates protein function by regulating protein post-phosphorylation, as it recognizes a specific motif of phosphorylated threonine or serine on the amino-terminal site of an adjacent proline (pSer/Thr-Pro), catalysing the isomerization of the peptide bond. Pin1 phosphorylates APP, Tau**,** some kinases and phosphatases which act on these proteins, hence regulating directly and indirectly the two key pathological hallmarks of AD. For example, Pin1 isomerizes p-

Thr231-Pro motif in Tau, restoring its ability to bind to microtubules and promoting their assembly. Also, Pin1 facilitates Tau dephosphorylation by calcineurin and non-amyloidogenic processing of APP [91, 111].

Aβ-induced oxidative stress activates a Tau kinase, glycogen synthase kinase 3β (GSK3β), leading to Tau hyperphosphorylation in most of its threonine and serine residues. Overexpression of GSK3β in neurons increases mitochondria activity, whereas a decrease in the activity of GSK3β attenuates mitochondrial activity and Nrf-2-mediated up-regulation of antioxidant enzymes. Inhibition of GSK3β signalling in a double-transgenic mouse model of AD reduced Aβ neuropathology and restored memory deficits. Aβ-induced oxidative stress also stimulates as an adaptive response the transcription of regulator of calcineurin 1 (*RCAN1*). RCAN1 decreases calcineurin levels, a p-Tau phosphatase, leading to hyperphosphorylation of Tau, and initiating mitophagy and shift of neuronal metabolism from oxidation to glycolysis [111, 112].

Oxidative stress - mediated production of $ONOO^-$ is a possible negative mediator for choline bioavailability, affecting acetylcholine (ACh) synthesis. Reduced synthesis of Ach neurotransmitter, increased catabolism of Ach by acetylcholinesterase (AChE) and de-sensitization of acetylcholine receptors is the oldest hypothesis on AD (cholinergic hypothesis), on which most current therapies are based [inhibitors of cholinesterase (ChEIs), e.g., rivastigmine, tacrine (THA), and galantamine (GNT)]. Inadequate cholinergic neurotransmission negatively affects neuronal signal transmission, cell survival and proliferation and promotes distal, retrograde nerve fibre degeneration. ChEIs inhibit the catabolism of ACh, increasing its concentration in the synapses, thus enhancing cognitive function. AChE is associated with the hallmarks of AD neuropathology; formation of β-amyloid plaques and neurofibrillary tangles [17, 113].

Overall, oxidative stress is considered a major aspect and a relatively early event in AD pathogenesis, as demonstrated by increased oxidative stress markers. For example elevated protein oxidation/nitration (protein carbonyls and 3-nitrotyrosine), mitochondrial and nuclear DNA oxidation (8-OHdG, 8-OHA, 5-OHU, 5-hydroxycytosine, 2,6-diamino-4-hydroxy-5-formamidopyrimidine, 4,6- diamino-5-formamidopyrimidine) products, protein-bound–HNE, MDA and isoprostane were found in the brains plasma, urine, and cerebrospinal fluid of subjects with AD, even with early mild cognitive impairment [2, 16, 24, 27, 28, 33, 90, 92, 114-116].

5.1.2. Antioxidant Capacity of Saffron and its Bioactive Constituents in Alzheimer's Disease

Administration of saffron or its bioactive constituents can reverse memory deficits in different behavioural tasks, and positively affect learning behaviour, recognition, spatial memory, and LTP in animal models and clinical studies.

5.1.2.1. Inhibitory Effects of Saffron on Aβ Fibrillogenesis

The anti-amyloidogenic properties of saffron were elucidated in a study on the inhibitory effects of methanolic saffron extract, trans-crocin-4 and DMCRT on $A\beta_{1\text{-}40}$ fibrillogenesis. An important observation was that methanolic saffron extract and DMCRT impeded the creation of amyloid fibrils in a concentration- and time-dependent manner, whereas *trans*- crocin-4 exhibited stronger inhibitory activity on Aβ- aggregation at low concentrations. In a study in which SH-SY5Y neuroblastoma cells were used as a cellular model of AD, co-incubation Aβ with crocins inhibited Aβ assembly in a concentration-dependent mode.

Crocins might prevent amyloid fibril formation by binding via their hydrophobic carotene backbone, shielding and stabilizing the central aggregation-prone hydrophobic cluster ($A\beta_{17\text{–}21}$), hence preventing conformational transition from an a-helix or random coil structure to a β-sheet, that is essential in generation of amyloidal structures. The central hydrophobic core is the main component of amyloid plaques, as found in the brain of AD patients. After incubation with crocins, $A\beta_{42}$ develops an a-helix structure that is a non-amyloidogenic conformation. Crocins interact with the C-terminal region ($A\beta_{28\text{–}42}$), which also has a probability for β-sheet structure formation, hence preventing interaction with other monomers, formation of paranuclei and subsequently amyloid fibrils. Also, crocins might prevent Aβ aggregation by interacting via their sugar units with molecules of water in the adjacent environment of Aβ peptides and/or by interacting via the alcohol groups of sugars with the polar ends of Aβ monomers. [16, 69, 86, 117-119].

CRT also inhibited Aβ fibrillization, stabilized Aβ oligomers, preventing their conversion to fibrils and destabilized pre-formed Aβ fibril assemblies. The negatively charged carboxyl groups and the hydrophobic hydrocarbon chain of CRT possibly interact with positively charged amino acids (lysine 16 or 28) and hydrophobic sequence at the C-terminal domain (isoleucine 31-valine 40) in Aβ, respectively. The Aβ C-terminal domain is important in generation of stable Aβ oli-gomers [119-121].

Moreover, CRT improved the ability of monocytes, which have the same myeloid origin with microglial cells, to degrade $A\beta_{42}$ via the lysosomal protease, cathepsin B [121].

5.1.2.2. Inhibitory Effects of Saffron on Tau Aggregation

Tau- directed therapeutic strategies focus mainly on inhibitors of tau hyperphosphorylation, anti-aggregation drugs, and microtubule stabilizing drugs [88].

Crocins in *in vitro* experiments inhibited the aggregation of human tau proteins, by decreasing the β-structure/random coil ratio of tau. The partial negative charge of carbonyl groups in crocins might be responsible for their interaction with positive amino acids, e.g. arginine and lysine, located in hexapeptide aggregation cores of protein and implicated in the abnormal self-assembly of tau into fibrils. Hence, crocins might impair self-assembly during nucleation and elongation of fibrils. β-D-gentiobiosyl sugar moieties at the ends of the polyene hydrocarbon chain of crocins augment the total hydrodynamic radius of tau, consequently inhibiting the necessary hydrophobicity for β- sheet formation of the aggregates [87]. Also, crocins enhance tubulin polymerization and microtubule nucleation rate in a concentration dependent manner, by interacting with these structures, which are normally tau targets [122, 123].

5.1.2.3. Modulation of Cholinergic Neurotransmission by Saffron

As aforementioned inhibition of acetylcholine breakdown is one of the primary therapeutic approaches for AD treatment, since cholinergic alterations are correlated with cognitive defects. Aqueous methanolic saffron extract might protect against AD by moderating AChE activity, hence improving cholinergic transmission and inhibiting Aβ aggregation. Inhibition of AChE was observed for saffron's constituents in the order safranal > CRT ≥ DMCRT, as IC50 values were calculated 21.09, 96.33, and 107.1 μM, respectively. CRT and DMCRT were found to bind concurrently to catalytic and peripheral anionic sites of AChE, presenting a mixed-type of inhibition in kinetic analysis [113]. The inhibitory effects on AChE were also demonstrated in a study that used a *Drosophila* model of Parkinsonism, which will be discussed next [124].

5.1.2.4. Reduction of Glutamatergic Synaptic Transmission by Saffron

NMDARs-dependent LTP at hippocampal synapses is a form of activity-dependent synaptic plasticity, implicated in memory and learning, hence blockade of NMDARs by antagonists or channel blockers can inhibit the induction of hippocampal LTP and impair the spatial cognition. Administration of 30-40% ethanol (10 ml/kg) prior to a learning trial impaired memory acquisition and retrieval both in step through and step down passive avoidance tests, by inhibiting synaptic responses induced by NMDARs stimulation, thus suppressing LTP in the dentate gyrus. Saffron alcohol extract alone did not have any effect on the induction of LTP, as assessed by memory registration, consolidation or retrieval. On the other hand, it abated ethanol-induced impairment of memory registration both in step through and step down passive avoidance tests and the ethanol-induced impairment of memory retrieval in step down passive avoidance test by antagonizing ethanol LTP-blocking effect in hippocampal dentate gyrus.

Intra-cerebro-ventricular injection or oral administration of crocins did not alter either NMDAR-mediated or non-NMDAR -mediated synaptic potential, thus had no effect on memory acquisition in normal mice in both step through and step down passive avoidance tests. However, when crocins were given before ethanol treatment, they improved the ethanol-induced memory deficit and impairment of learning in passive avoidance performance tasks. Crocins reduced the inhibitory effect of ethanol on NMDARs-dependent LTP, thus protected against ethanol-impairment of hippocampal synaptic plasticity. Intra-cerebro-ventricular injection of CRT also antagonized the LTP-blocking effect of ethanol in the rat hippocampus, but higher doses than crocins were required, in order to achieve a more significant effect. Neither free gentiobiose nor glucose mimicked the activity of crocins, implying that gentiobioses attached to the fatty acid chain are critical for crocins to exert their biological activity. Picrocrocin, the most abundant component in saffron did not affect the ethanol-impairment of memory acquisition and retrieval in step through and step down passive avoidance tests at doses up to 50-200 mg/kg.

There are studies suggesting that saffron extracts and CRT exhibit a strong affinity for phencyclidine binding site of NMDARs and σ1Rs (sigma 1 receptors), whereas crocins and picrocrocin were not as effective. Binding of saffron extract and CRT to NMDARs could lead to an antagonistic effect, as known ligands for the PCP binding site block the channel pore of NMDARs, partially explaining the effects of saffron on symptoms from the CNS or behavioural problems.

In another study on rats crocins counteracted schizophrenia-like behavioural deficits (hypermotility, stereotypies, ataxia, social isolation) and cognition impairment (deficits in recognition memory), evoked by ketamine, a non-competitive NMDAR antagonist. Treatment with crocins by itself did not alter these behaviours. Ketamine acts via blocking the activity of NMDARs located on GABA interneurons (γ-aminobutyric acid), leading to disinhibition of neural activity in limbic structures and subsequent excessive glutamate and dopamine release in the prefrontal cortex and limbic regions. It is postulated that crocins exerted their beneficial action on ketamine-induced behavioural deficits by reducing glutamate levels and glutamatergic synaptic transmission [125, 126].

σ1Rs are concentrated in certain regions of CNS and modulate the activity of several ion channels and G-protein-coupled receptors [127], including Ca^{2+} channels [56], NMDARs [75, 128], dopamine and acetylcholine receptors [113, 129], thus are implicated in various diseases, such as depression, schizophrenia [126, 130], and cocaine addiction [126, 130]. Also, σ1Rs ligands inhibit the presynaptic release of excitotoxic amino acids during ischemia [113], indicating that σ ligands, such as saffron extracts and CRT could serve as neuroprotective agents [103, 129].

5.1.2.5. Restoration of Cellular Antioxidant Defences by Saffron

An important mechanism by which saffron and its constituents protect against neurodegeneration is restoration of neuronal antioxidant defence content and activity, such as GSH, GPx, GR, GST, SOD and catalase, hence counteracting oxidative modifications of cellular organelles and molecules.

The antioxidant effect of saffron extract and crocins against brain, liver, and kidney damage during chronic -stress induced oxidative stress was investigated in a study on male Wistar rats, which were restrained daily for six hours for a total of 21 days, without access to food and water. Stressed animals demonstrated higher content of MDA, as assessed by TBARS, higher activities of GR, GPx, and SOD enzymes in brain, liver and kidneys, as an adaptation in chronic stress and lower total antioxidant capacity, as assessed by FRAP, compared to the non-stressed animals. In the stressed group higher levels of corticosterone were demonstrated, a finding that could be supported by the notion that glucocorticoids may be implicated in chronic-stress induced oxidative damage and impairment of antioxidant defences in different brain regions, including hippocampus, cortex, and cerebellum, thus possibly implicated in neuronal damage. Chronically elevated glucocorticoids inhibit neuro-

genesis and cause hippocampal volume loss and atrophy of dendrites in hippocampal CA3 pyramidal neurons. The previous changes from clinical perspective are related to stress-mediated impairment in spatial learning and memory. Pretreatment with saffron extract and crocins reversed the previous changes in the stressed animals as compared to control groups, by boosting the activity of detoxifying cellular mechanisms, thus protecting vital organs with high oxygen turnover against oxidative stress. Saffron was more efficient than crocins, probably due to synergy of its various antioxidant components, such as crocins, CRT, safranal, and flavonoids. Saffron and crocins also deterred the oxidative stress damage in hippocampus and impairment of learning and memory, thus these substances may be useful in alleviation of cognitive deficits [83, 131].

Intra-cerebro-ventricular injection of streptozotocin (STZ), in a sub diabetogenic dose in male Wistar albino rats, caused prolonged deprivation of brain glucose and energy metabolism and induced oxidative stress. STZ is a naturally occurring glucosamine-nitrosourea which is particularly toxic to the insulin B-cells of pancreas and insulin receptors. Decreased cerebral glucose uptake and energy metabolism in STZ-lesioned rats was complicated with severe and progressive memory deterioration and poor learning performance, due to inadequate hippocampal choline acetyltransferase content, mimicking behavioural problems and memory dysfunction in age-related neurodegenerative diseases. This observation is of particular importance as there is accumulating evidence that glucose hypometabolism and impaired insulin signalling in CNS are early and persistent events in AD. The behavioural features of STZ-lesioned rats were correlated with STZ –induced oxidative stress, as demonstrated by elevated MDA content, depletion of GSH, and total thiol pool and depressed GPx activity in brain tissues. It has been postulated that oxidant radicals inactivate GPx. Oral administration of crocins improved significantly cognitive performance, attenuating memory deficits in STZ-injected mice in passive avoidance test, in parallel with restoring GPx activity, thus reducing lipid peroxidation and MDA pool and replenishing total thiol content, verifying their antioxidant potential [132, 133]. *Striatum* was chosen because it is particularly susceptible to oxidative stress damage, due to depressed endogenous levels of antioxidant defence [105, 134-137].

Cerebral hypoperfusion produces massive amounts of ROS which overwhelm brain's antioxidant machinery, especially in the cortex and hippocampus. Saffron extract (50, 100, 250 mg/kg) and crocins (5, 10, 25 mg/kg) ameliorated cognitive deficits induced by chronic cerebral hypoperfusion, a neuroprotective effect that was attributed to their antioxidative properties [53]. Mice exposed to safranal intraperitoneally (72.75 mg/kg, 145.5 mg/kg, 363.75 mg/kg, 727.5 mg/kg) also exhibited significant restoration of hippocampal antioxidant capacity, as assessed by FRAP and total –SH content, both of which were compromised during cerebral ischemia-reperfusion injury. The previous findings explain the significant and dose-dependent decline of MDA levels in hippocampus of rats receiving safranal [36].

In vitro studies on neuronally differentiated pheochromocytoma cells (PC-12) demonstrated that stress stimuli (*e.g.* serum/glucose deprivation, hypoxia) trigger cellular oxidative stress, as demonstrated by a decline in intercellular levels of GSH and SOD activity. Treating cells with crocins exerted a significant neuroprotective effect, by inhibiting the formation of peroxidised lipids and maintaining neurons' morphology. The effects of crocins were attributed to the restoration of the activity and expression of SOD, GR and γ-glutamyl-cysteinyl synthase (γ-GCS), the rate-limiting enzyme of GSH synthesis, thus restoring GSH pool.

Acrolein rapidly binds and depletes cellular anti-oxidant enzymes (SOD and GPx), GSH, total and nuclear levels of Nrf2. Also, it is correlated with mitochondrial dysfunction, as demonstrated by decreased MTP, inhibition of the respiratory machinery, e.g. complexes I and II, pyruvate dehydrogenase, and a- ketoglutarate dehydrogenase, leading to impaired cellular respiration, energy depletion, ROS generation, DNA damage, lipid peroxidation, and cellular apoptosis [116, 138-142]. Oral administration of acrolein, into mice, produactivatedced the the MAPK/ERK (Extracellular signal–regulated kinase) signalling cascade, as verified by increased levels and phosphorylation of upstream kinases ERK1/2, c-JNK and p-38 kinases, leading to a significant decline in GSH pool and increase in MDA content, Aβ deposition and tau phosphorylation in cerebral cortex. Co-administration of crocin modulated MAPKs signalling pathways, limiting MDA pool, Aβ levels and p-tau phosphorylation, thus preventing neuron apoptosis [142].

5.1.2.6. Inhibition of Cellular Apoptosis or Death by Saffron

Crocins might exert neuroprotection *via* inhibiting Aβ-induced neuronal apoptosis. Aβ hippocampal injection in adult male albino Wistar rats instigated cell death by autophagy, as indicated by increased concentrations of beclin-1 and ratio LC3 (Microtubule-associated proteins 1A/1B light chain 3B)-II/LC3-I or apoptosis, both intrinsic and extrinsic, as demonstrated by a pro-apoptotic shift in balance of Bax and Bcl-2 and increased levels of cleaved caspase-3. Beclin-1 plays a main role in the initiation of autophagy and ratio LC3 -II/LC3-I is an indicator of autophagy as LC3-I is processed to LC3-II during the formation of autophagosome. Intra-hippocampal and intra-peritoneal administration of crocins significantly decreased Bax/Bcl-2 ratio and activated caspase-3 in a dose-dependent mode. On the contrary, they did not affect autophagy, as indicated by insignificant alterations in LC3 -II/LC3-I ratio, and beclin-1. Also crocins in the presence of Aβ improved spatial memory, as indicated by enhanced escape latency, travelled distance and time spent in target quadrant, when compared to β-amyloid intra-cerebro--ventricular injection, which mimics behavioural alterations and spatial memory deficits in early to middle stages of AD. However, when crocins were administered alone, they did not alter any behavioural parameters [93].

In SH-SY5Y neuroblastoma cells Aβ reduced cell viability *via* significant increases in activated caspase 3 and phosphorylated (Thr183/Tyr 185) JNK, which represent apoptotic markers and may be involved in early signalling pathways implicated in *Aβ* toxicity. On the contrary, co-incubation of cells with different concentrations of crocins (1-50 μM), increased significantly cell viability in a dose-dependent mode, inhibiting Aβ- mediated cell apoptosis.

Exposure of PC-12 cells to environmental stress stimuli, such as serum/glucose deprivation and hypoxic conditions, triggered necrosis or apoptosis by activation of nSMase and ceramide generation. Ceramide is implicated in cellular apoptosis via diverse signalling pathways, among of which is translocation to cytoplasm of cytochrome c and AIF proteins. Intracellular GSH exhaustion due to oxidative stress, which apart from a major antioxidant, is a potent inhibitor of NSMase, sensitizes cells to various death-inducing agents, also via the ceramide pathway [108, 109].

Treatment of PC-12 cells with crocins, as aforementioned replenished GSH levels, obviating NSMase activation, ceramide production and ceramide -mediated cell death. However, GSH replenishment should be an early event, in order to maintain ceramide levels below the threshold. Once ceramide exceeds threshold levels, GSH may no longer obstruct ceramide-induced neuronal cell death [143-145]. Crocins modulated in a concentration-dependent manner the expression of Bcl-2 proteins in the presence of TNF-α; they suppressed the expression of pro-apoptotic proteins Bcl-XS and caspase 8, an initiator- caspase, leading to the execution phase of apoptosis, via activation of caspase-3. Also, crocins restored the anti-apoptotic Bcl-xL (B-cell lymphoma-extra large), leading to a marked diminution of TNF-α-mediated mitochondrial release of cytochrome c [144, 146]. The previous antioxidant and cytoprotective effects of crocins were superior compared to those of a-tocopherol [143]. Retinoic acid, a structurally related retinoid molecule did not have any positive effect on PC-12 cells [144].

5.1.2.7. Clinical Studies of Saffron in Patients with Alzheimer's Disease

The efficacy of saffron and its constituents in mitigating the AD-related manifestations were demonstrated in clinical studies. In a double-blind and randomized study on patients with mild-to-moderate AD, saffron treatment (15 mg twice per day) was as effective as donepezil (5 mg twice per day), a cholinesterase inhibitor, after 22 weeks in improving cognitive function. In addition, patients receiving saffron did not experience the devastating vomiting, dizziness, fatigue, and nausea which were significantly more frequent in the donepezil group. On the contrary, patients receiving saffron had slightly more cases of dry mouth and hypomania [147, 148]. The results were also encouraging concerning mild cognitive impairment, since patients treated with saffron exhibited better performance, as assessed by Mini-Mental State Examination (MMSE). Moreover, MRIs (Magnetic resonance imaging) showed a small volume difference on the left inferior temporal gyrus, which is adjacent to medial temporal lobe and hippocampus [149].

In a randomized double-blind parallel-group research on patients with moderate to severe AD for at least 6 months (MMSE score of 8–14), one year administration of saffron extract (30 mg/day) demonstrated comparable results with memantine treatment, an NMDAR antagonist, in terms of stabilizing or slowing the rate of cognitive deterioration. Depression is a frequent and challenging comorbidity in AD, with a high prevalence of up to 50%, and a negative impact on life quality and long-term prognosis) In addition to cognitive improvement, patients with AD may also benefit from the antidepressant properties of saffron, as treatment of mild to moderate depression with saffron capsules (30 mg/day) was equally effective with imipramine and fluoxetine (selective serotonin reuptake inhibitor/SSRIs). Crocins (30 mg/day) were also an efficient adjunctive treatment in major depressive disorder, when co-administered with an SSRI (sertraline 50 mg/day, fluoxetine 20 mg/day or citalopram 20 mg/day) [46, 69, 150-152].

5.2. Parkinson's Disease

5.2.1. Oxidative Stress in Parkinson's Disease

Parkinson's disease (PD) is the most prevalent age-related motoric neurodegenerative condition and the second most common age-related neurodegenerative disease after AD. Although the aetiology of the disease is not fully elucidated, inadequate defence against oxidative stress of dopamine-secreting neurons in the *substantia nigra pars compacta*, is complicated with profound cellular dysfunction and dopamine depletion, due to the presence of intracytoplasmic Lewy bodies. The latter are composed of fibrillar a-synuclein and are considered hallmarks of PD initiation and progress. a-synuclein is a presynaptic protein, which is prone to fibrillar aggregation due to its hydrophobic non-amyloid beta component domain. Approximately 60-80% of dopaminergic neurons are lost before the emergence of PD motor signs. Generation of ROS is exacerbated in PD due to neuroinflammation, dopamine degradation, GSH depletion, mitochondrial dysfunction, and disruption of normal cellular homeostasis of iron or Ca^{2+}.

Dopamine can act as metal chelator, since iron is accumulated in dopaminergic neurons of patients with PD, generating •OH *via* Fenton reaction. Also, it can be metabolized into 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the presence monoamine oxidase (MAO), a flavoprotein at the outer mitochondrial membrane. DOPAC can be oxidized by H_2O_2 to toxic metabolites, which subsequently destroy dopamine storage vesicles. Auto-oxidation of excess cytosolic dopamine itself to toxic electron-deficient dopamine-quinones or semiquinones can be a source of ROS. Dopamine- quinone species in the cell bind and covalently modify nucleophiles, including low molecular weight sulfhydryls, such as protein cysteinyl- residues, and GSH, whose normal function is necessary for cell survival.

Dopamine- quinone species alter the structure or function of proteins which are implicated in Parkinson's pathology, such as a-synuclein, parkin (E3 ubiquitin ligase), PARK7 (Parkinson disease protein 7)/DJ-1 (protein deglycase DJ-1), and UCH-L1 (ubiquitin carboxy-terminal hydrolase L1, a deubiquitinating enzyme), hence inhibiting the normal protein degradation by chaperone-mediated autophagy or proteasome. When dopamine- quinone species interact with α-synuclein monomer, they promote its conversion to the cytotoxic protofibril form, whereas when they interact with parkin or S-nitrosylation of parkin, result in inactivation of its E3 ubiquitin ligase activity. Parkin is involved in proteasome-mediated degradation of proteins, susceptible to aggregation and the loss of its activity is implicated in autosomal -recessive juvenile Parkinsonism. Also, parkin is local-

ized in the mitochondria, regulating mitochondrial respiration and activity, transcription and replication. Overexpression of parkin attenuated ceramide-mediated loss of dopaminergic neurons, when exposed to neurotoxins, via delaying mitochondrial swelling and subsequent release of cytochrome *c*, attenuating protein oxidation and lipid peroxidation and reducing a-synuclein. Also, parkin is recruited by defective mitochondria, implicated in mitochondrial autophagy. DJ-1 is a multifunctional cytosolic protein with antioxidant and transcriptional modulation characteristics. Mutations of *DJ-1* are implicated in rare cases of early onset Parkinsonism, inherited in an autosomal recessive manner. During oxidative stress DJ-1 translocates into the mitochondria to protect against oxidative stress, e.g. by facilitating the activity of complex I, restoring MTP and mitochondrial morphology and interacting with PINK1 (PTEN-induced putative kinase 1). Also, DJ-1 may translocate to the nucleus to activate transcription of *Mn-SOD***.** In patients with PD, mutations of *UCH-L*1 are associated with partial loss of the enzyme's catalytic activity, whereas UCH-L1 is extensively modified by methionine or cysteine oxidation, and carbonyl formation, in idiopathic PD and AD. Dopamine quinones inactivate dopamine transporter, which is implicated in dopamine re-uptake out of the synaptic cleft to the cytosol and tyrosine hydroxylase, which converts L-Tyrosine into L-DOPA, the precursor of dopamine using tetrahydrobiopterin and Fe^{2+} as cofactors. The presence of a- synuclein intracellular inclusions and oxidative stress inhibit autophagy, initiating a cycle in which dysregulation of autophagy increases ROS content and accumulation of ubiquitinated proteins [100, 129].

Also, subunits of complex I and complex III in mitochondrial electron transport chain are targeted by dopamine quinones, leading to mitochondrial dysfunction and energy depletion. Furthermore, dopamine quinones can transform to reactive aminochromes, whose redox-cycling generates superoxide, depleting cellular NADPH, and subsequently polymerize to neuromelanin, which accumulates in the nigral region of the brain. Neuromelanin released from dying neurons activates microglia, hence triggering neuroinflammation and increasing the sensitivity of dopamine neurons to oxidative stress-mediated cell death, exacerbating the neurodegenerative process [129, 153-156].

Mounting evidence suggests that direct generation of ROS might be correlated with compromised mitochondrial oxidative phosphorylation, due to deficiency in complex I at synaptic mitochondria. The defect is correlated with *PINK1*/PARK6 mutations, the second most common cause of autosomal recessive, early onset PD, after parkin mutations. PINK1 is a mitochondrial serine/threonine kinase with anti-apoptotic properties, localized in different regions, including the outer or inner mitochondrial membranes, and intermembrane space. Its mutations are associated with loss of dopaminergic neurons, due to abnormal mitochondrial morphology and integrity, reduced mitochondrial electrochemical gradient, deficient mitochondrial respiration, increased basal ROS generation and Ca^{2+} signalling, enhanced vulnerability to oxidative stress, opening of the voltage dependent mPTP and sensitization to apoptosis, during intense stimulation of the synapse. PINK1 most probably exerts its neuroprotective effect by phosphorylating specific mitochondrial proteins, such as mitochondrial heat shock protein TRAP1, proapoptotic serine protease Htr2a/OMI and parkin. Overexpression of parkin can rescue mitochondria dysfunction caused by PINK1 deficiency. In a-synuclein the N-terminal 32 amino acids contain cryptic sequence which can cause its targeting by mitochondria, also leading to inhibition of complex I [21, 100, 101, 129, 153-155, 157].

Altered Ca^{2+} homeostasis is implicated in Parkinson's disease via disruption of mitochondrial activity and generation of oxidative stress. Dopaminergic neurons in *substantia nigra pars compacta* are autonomously active and engender broad, slow action potentials in the absence of synaptic input, through sustained activation of L-type Cav 1.3 Ca^{2+} channels, allowing influx of Ca^{2+} into the cytoplasm. This pacemaking activity is essential for maintenance of ambient dopamine pools in regions innervated by these neurons. Altered Ca^{2+} homeostasis can shift dopamine concentrations in the cytosol to a toxic range with L-DOPA loading. Small oligomers of α-synuclein dysregulate Ca^{2+} and calpain, an important Ca^{2+}-activated protease, which in turn cleaves α-synuclein, producing a truncated form, prone to aggregate and form fibrils. Ca^{2+} compromised homeostasis and fibril aggregation can lead to Ca^{2+}-triggered conformational change and opening of mPTP, causing mitochondrial swelling and consequent rupture of the outer membrane, allowing Ca^{2+} cytosolic influx, further stressing nearby mitochondria [100, 129].

The low levels of GSH content and the decreased activity of catalase and its dependent enzymes (GPx, GR and GST) may be important pre-symptomatic drivers of PD. Catalase and GPx detoxify H_2O_2 to H_2O and GST catalyses detoxification of catecholamines (o-quinone) oxidized metabolites; thus, inadequacy of these enzymes is implicated in neurodegeneration. [2, 16, 153-156, 158-161].

4-HNE is of particular importance in PD, as it is highly generated in surviving dopamine neurons in *substantia nigra* of subjects with PD. It inactivates mitochondrial ALDH, by forming a Michael adduct or Schiff base, inhibiting the catabolism of dopamine, and leading to increased 3,4-dihydroxyphenylacetaldehyde, an endogenous neurotoxin. ALDH is an enzyme implicated in detoxification of endogenous (4-HNE, MDA) and xenogenic/ environmental (acrolein) aldehydic products [162].

5.2.2. Antioxidant Capacity of Saffron and its Bioactive Constituents in Parkinson's Disease

The effect of CRT in mitigating oxidative stress was demonstrated in a 6- hydroxydopamine (6-OHDA) model of rat Parkinsonism. Accumulation of endogenous 6-OHDA, which is a neurotoxin that selectively destroys dopaminergic and noradrenergic neurons in the brain, has been identified in patients with PD. Treatment of male Wistar rats with unilateral intrastriatal injection of synthetic 6-OHDA inhibited the activity of antioxidant enzymes (catalase, SOD GPx, GR, GST) and exhausted dopamine pool. 6-OHDA engenders ROS through oxidation, and inhibition of mitochondrial complex I. The increased oxidative burden was reflected by increase in TBARS and nitrite levels, a stable NO metabo-

lite, in the hippocampus of 6-OHDA lesioned rats. From behavioural perspective, the lesion group demonstrated significant cognitive deficits, attenuation in average speed and distance travelled [163].

Pre- treatment with CRT (25, 50 and 75 μg/kg) for 7 days prior to 6-OHDA administration attenuated TBARS content, while preserving levels of dopamine and antioxidant enzymes, especially GPx, that exhibits relatively high activity in *striatum* and *substantia nigra*. Histopathologic findings in the *substantia nigra*, demonstrated that CRT could protect neural cells from the detrimental effects of 6-OHDA. Rats treated with CRT showed compelling and dose-dependent increase in the speed and distance covered and recovery in locomotion. The study demonstrated that CRT was able to partially shield against degeneration of dopaminergic neurons by 6-OHDA, via suspending accumulation of ROS [164].

Treatment with crocins also exerted significant antioxidant properties on 6-OHDA lesioned rats, as demonstrated by decreased TBARS in hippocampus and reduced nitrite levels (nitrosative stress), probably due to suppression of NOS activity and NO generation. Crocins affected positively 6-OHDA induced- memory impairment [163].

The neuroprotective properties of saffron methanolic extract and crocins were also demonstrated in an idiopathic *Drosophila* Parkinson's disease model, induced by rotenone (ROT), a mitochondrial toxin. ROT can interfere with mitochondrial electron transport chain, inhibiting the transfer of electrons from iron-sulphur centres in complex I to ubiquinone, thus increasing the reduction state of the complex and the leakage of electrons, which interact with oxygen to form superoxide. ROT can have deleterious effects in any organ and tissue, as it is a lipophilic molecule, a characteristic enabling it to readily cross biological membranes and the blood–brain barrier, independently of any receptor or transporter. In 2011, a US National Institutes of Health research linked ROT to PD in farm workers, who use ROT as an organic pesticide [129, 165]. Paraquat (1,10 -dimethyl-4,40 -bipyridinium dichloride) (PQ) is a commonly used herbicide that has been linked to human PD, due to selective and dose-dependent loss of dopaminergic neurons in the *substantia nigra* and reduction in dopamine levels. PQ accumulates in mitochondria, acting as a potent redox cycler, converting ROS which interact with molecular oxygen to generate several ROS. Also, PQ can interact with glutamate, leading to depolarization of NMDARs and activation of non-NMDAR channels, causing excitotoxicity via Ca^{2+} efflux. The previous activate NOS in dopaminergic neurons, resulting in NO up-regulation, and further mitochondrial dysfunction. Exposure of flies to ROT and PQ can result in Parkinson's disease- like manifestations and pathology, linked with mitochondrial dysfunction and oxidative stress [129].

ROT was applied to flies to induced oxidative stress, mitochondrial dysfunction, neurotoxicity, locomotor phenotype dysfunction, and mortality. Saffron methanolic extract and crocins restored the activity of antioxidant enzymes (SOD, catalase, GST, thioredoxin reductase), thus augmenting GSH and total thiol levels and diminishing oxidative markers (ROS, hydroperoxides and NO) in the head and body of flies in a concentration-dependent manner. ROT-induced inhibition of mitochondrial complexes I–III was markedly mitigated by saffron methanolic extract and crocins.

In ROT-treated flies the activity of AChE was significantly elevated in their head (40%) and body (37%), whereas co-exposure with saffron methanolic extract and crocins restored the activity of AChE (35-31% for saffron methanolic extract and 19-21 % for crocins), in a concentration-dependent manner, hence mitigating cholinergic dysfunction. Clinically, the rescue of dopaminergic neurons with saffron or crocins in the ROT-challenged fly model led to a significant restoration of neurotransmitter levels, thus improving locomotor ability of flies by up to 25% and reducing ROT-induced mortality. The median and maximum life span of flies is 80 and 94 days, respectively. Feeding with a diet supplemented with saffron methanolic extract or crocins extended maximum life span by 10-20%. Saffron methanolic extract and crocins also provided adequate prophylaxis when flies were exposed to PQ. Taken together, the neuroprotective effects of saffron against neurotoxins may be largely attributed to modulation of endogenous levels of oxidative defence mechanisms [124].

Safranal was also able to inhibit ROT- induced cell death and ROS production in a dose-dependent way in an *in vitro* PD model of dopaminergic neurons. The neuroprotective effects of safranal were attributed to inhibition of expression of kelch-like ECH-associated protein 1 (Keap1) and up-regulation of expression of the transcription factor Nrf2, which induced the expression of several antioxidant enzymes, e.g. GST, GCLc, NQO1 and HMOX-1 [166].

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) imparts its toxicity on dopaminergic cells, via conversion to the active toxin, MPP^+ (1-methyl-4-phenylpyridinium), which concentrates within the mitochondria. MPP^+ inhibits the activity of complex I, hence involved with ATP depletion, oxidative stress, apoptotic cell death, and glutamatergic excitotoxicity [167, 168]. In an acute MPTP mouse model, intraperitoneal injection of MPTP depleted dopaminergic cells in the *substantia nigra pars compacta* and dopaminergic amacrine cells in the retina, as assessed by tyrosine hydroxylase immunochemistry. The deleterious effects of MPTP were attributed to mitochondrial dysfunction and ROS-induced damage to key proteins of dopaminergic cells, including tyrosine hydroxylase. Saffron pre-treatment protected dopaminergic cells via increasing total brain antioxidant activity, hence repressing ROS formation, lipid peroxidation, and caspase-3 activation [169].

In an *in vitro* MPP^+ -induced PD model of PC12 cells, MPP^+ reduced cell viability in a dose- and time-dependent mode, whereas crocins significantly attenuated MPP^+-induced cell injury and apoptosis, even if treatment with crocins was delayed for 6 hours after MPP^+- injury. The cytoprotective effects of crocins were correlated with mitigation of MPP^+-prompted mitochondrial dysfunction, as determined by preservation of MTP and ATP generation and attenuation of mitochondrial swelling and cytochrome c release.

MPP^+ exposure was complicated with morphological changes of ER and triggered ER stress, as verified by Ca^{2+} release, disrupting Ca^{2+} homeostasis, and expression of ER stress-related proteins, such as GRP78 (78 kDa glucose-regulated protein)/ HSPA5 (heat shock 70 kDa protein 5), CHOP (DNA damage-inducible transcript 3, C/EBP homologous protein)/ GADD153 and caspase-12. GRP78/ HSP70 is a molecular chaperone located in the lumen of ER that binds nascent secretory proteins, aiding their proper conformational maturation, subsequent folding and oligomerization and retaining misfolded proteins in the ER. ER stress is implicated in PD, as it can be triggered in the presence of aberrant protein folding and degradation. CHOP is a protein of C/EBP family of transcriptional regulators, implicated in growth arrest and apoptosis after DNA damage or ER stress. It can induce apoptosis *via* activation of Ero1 (ER oxidoreductin 1), that causes Ca^{2+} release from ER or GADD34/ PPP1R15A (Growth arrest and DNA damage-inducible protein/ Protein phosphatase 1 regulatory subunit 15A). Also, CHOP could sensitize cells to ER stress *via* decreasing GSH activity, stimulating ROS generation, and regulating Bcl-2 family proteins. In case of severe or prolonged ER stress, it can sensitize mitochondria to trigger apoptosis, *via* caspase-12, an ER-resident caspase, which subsequently activates caspase-3, the executer caspase of apoptosis.

Crocins attenuated morphological changes of ER and suppressed ER stress, thus preventing Ca^{2+} release from ER and inhibited expression of CHOP and binds to caspase-12 and endoplasmic reticulum chaperones [170].

5.3. Oxidative Stress and Schizophrenia

Schizophrenia affects approximately 1% of the population and it is considered a serious mental health problem affecting the personal and social existence. The aetiology of schizophrenia is largely unknown, yet several hypotheses position the pathology of schizophrenia to the variations of a network of genes [171]. Yet, another hypothesis puts in the centre the role of oxidative stress as a possible reason for the pathophysiology of schizophrenia. The brain is very susceptible to the effects of oxidative stress due to the increased oxygen consumption and the high metal ion (iron, zinc, copper, manganese) presence in it [171, 172]. At the same time the brain lacks anti-oxidant defensive mechanisms, which makes it vulnerable to ROS. Some of the defence mechanisms include the removal of ROS, the inhibition of ROS creation and the binding of metal ions required for ROS catalysis. Another important anti-oxidant mechanism is Nitrogen Oxide (NO), which is also a signalling molecule. NO is a free radical, which plays a role in both the anti- as well as the pro-oxidant machinery [171]. The role of oxidative stress in schizophrenia has been revealed through clinical trials, which have been controversial as far as the role of oxidative stress in schizophrenia is concerned. In a recent report it has been shown that there is an increase in the levels of lipd peroxidation products and NO and at the same time superoxide dismutase was found to be decreased [171]. In a post-mortem schizophrenic cohort, a 40% depletion of GSH has been reported [173, 174]. Finally, known plasma anti-oxidants were significantly lower in schizophrenia, as compared to healthy controls, while thioredoxin levels were higher [171, 173, 175]. In post-mortem schizophrenic cohort, a 40% depletion of GSH has been previously reported [173, 174]. Further on, known plasma anti-oxidants are reported to be significantly lower in schizophrenia as compared to healthy controls, while thioredoxin was found to be higher [171, 173, 175]. Finally, memory impairment is a frequent problem in schizophrenia. Saffron aqueous extract and crocin were evaluated in a 12 week period in a group of patients with schizophrenia, while they received their normal treatment, to evaluate their safety. The study revealed that both substances in doses of 15 mg twice daily were safely tolerated, without any significant changes in hematologic or biochemical indices, whereas saffron aqueous extract could prevent olanzapine-induced metabolic syndrome [176, 177].

CONCLUSION

Oxidative stress arises due to disturbed equilibrium between ROS generation and elimination or neutralization processes, in favour of the former. Overproduction of ROS *via* mitochondrial dysfunction and inactivation of respiratory-chain enzymes lead to respiratory burst and augmented the activity of various oxidases during environmental stress or inflammatory stimulus and/or exhaustion or impairment of cell's antioxidant capabilities.

Abnormally elevated ROS levels have deleterious effects on the CNS, as neuronal cells are post-mitotic cells, thus are incapable of proliferation and therefore, are particularly vulnerable to oxidative stress, leading to irreversible neuronal damage. Overall, it is well documented that administration of saffron or its bioactive constituents has neuroprotective properties, can reverse memory deficits in different behavioural tasks, and positively affect learning behaviour, recognition, spatial memory, and LTP in animal models and clinical studies. Finally, one of the main benefits for the administration of saffron in the cases of neurodegenerative diseases is the lack of side or adverse effects.

Thus, the use of saffron as a therapeutic agent in neurodegenerative diseases is very promising and further research is required for the validation of its uses.

LIST OF ABBREVIATIONS

$^1O_2$	=	Singlet oxygen
5-OHU	=	5-hydroxydeoxy uridine
6-OHDA	=	6- hydroxydopamine
8-OHA	=	8-hydroxydeoxy adenosine
8-OHdG	=	8-hydroxydeoxy guanosine
8-oxoG	=	8-dihydro-8-oxoguanosine
ABAD	=	A-binding alcohol dehydrogenase
ACh	=	Acetylcholine
AChE	=	Acetylcholinesterase
AD	=	Alzheimer's disease
ADH	=	Alcohol dehydrogenase
AGEs	=	Advanced glycation end products

AIF	=	Apoptosis inducing factor
ALDH	=	Aldehyde dehydrogenase
ALS	=	Amyotrophic lateral sclerosis
AMPA	=	2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) proprionate
Apaf- 1	=	Activating factor 1
ApoD	=	Apolipoprotein D
APP	=	Amyloid precursor protein
ATP	=	Adenosine 5′-triphosphate
Aβ	=	Amyloid β
Bcl-2	=	B-cell lymphoma 2
Bcl-xL	=	B-cell lymphoma-extra large
BHT	=	Butylated hydroxytoluene
-CH_2	=	Methylene groups
ChEIs	=	Cholinesterase inhibitors
CHOP	=	DNA damage-inducible transcript 3, C/EBP homologous protein
CNS	=	Central nervous system
COX	=	Cyclooxygenase
CRT	=	Crocetin
CypD	=	Cyclophilin D
DMCRT	=	Dimethylocrocetin
DOPAC	=	3,4-dihydroxyphenylacetic acid
DPPH	=	1,1-diphenyl-2- picryl-hydrazyl
eNOS	=	Endothelial NOS
ERK	=	Extracellular signal–regulated kinase
Ero1	=	ER oxidoreductin 1
FFA	=	Free fatty acid
FRAP	=	Ferric Reducing Activity of Plasma
FRAP	=	Ferric reducing power activity
GABA	=	γ-aminobutyric acid
GADD34/	=	Growth arrest and DNA damage-inducible
GClc	=	Glutamate-cysteine ligase catalytic
GCLM	=	Glutamate-cysteine ligase modifier
GNT	=	Galantamine
GPx	=	Glutathione peroxidase
GR	=	Glutathione reductase
GRP78/	=	78 kDa glucose-regulated protein/ heat
GSH	=	Glutathione
GSK-3β	=	Glycogen synthase kinase-3β
GSSG	=	Glutathione disulphide
GST	=	Glutathione-S-transferase
H_2O_2	=	Hydrogen peroxide
HCOR	=	Aldehydes
HMOX1	=	Heme oxygenase-1
HNE	=	4-hydroxynonenal
HNO_2	=	Nitrous acid
HO_2	=	Hydroperoxyl radical
HOBr	=	Hypobromous acid
HOCl	=	Hypochlorous acid
HOO•	=	Perhydroxyl radical
hPreP	=	Human Presequence Protease
HSPA5		shock 70 kDa protein 5
HTCC	=	4-hydroxy-2,6,6-trimethyl-1-carboxaldehyde-1-cyclohexene
HVA	=	Homovanillic acid
ICAM-1	=	Intracellular adhesion molecule-1
iGluRs	=	Ionotropic receptors
IL	=	Interleukin
IL-6	=	Interleukin-6
iNOS	=	Inducible NOS
JNK	=	c-Jun N-terminal kinase
Keap1	=	Kelch-like ECH-associated protein 1
KSR	=	Kinase suppressors of Ras
L•	=	Lipid radical
LC3	=	Microtubule-associated proteins 1A/1B light chain 3B
LDH	=	Lactate dehydrogenase
LOO•	=	Lipid peroxyl
LOO•	=	Lipid peroxyl radical
LOX	=	Lipoxygenase
LTP	=	Long term potentiation
MAO	=	Monoamine oxidase
MAPK	=	Mitogen-activated protein kinases
MAPKK6	=	Mitogen-activated protein kinase 6
MCP-1	=	Monocyte chemotactic protein-1
MDA	=	Malondialdehyde
MDR	=	Multidrug resistance
MetS•	=	Methionine sulfuranyl radical
MetSOx	=	Methionine sulfoxide

mGluRs	=	Metabotropic receptors
MMSE	=	Mini-Mental State Examination
MPP^+	=	1-methyl-4-phenylpyridinium
MPTP	=	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mPTP	=	Mitochondrial permeability transition pore
MRI	=	Magnetic resonance imaging
MSR	=	Methionine sulfoxide reductase
mtDNA	=	Mitochondrial DNA
MTP	=	Mitochondrial transmembrane potential
N_2O_3	=	Dinitrogen trioxide
N_2O_4	=	Dinitrogen tetraoxide
NAD	=	Nicotinamide adenine dinucleotide
NADPH	=	Nicotinamide adenine dinucleotide phosphate
NFT	=	neurofibrillary tangles
NMDA	=	N-methyl-D- aspartate
NMDAR	=	N-methyl-D-aspartate-type glutamate receptor
nNOS	=	Neuronal NOS
NO^-	=	Nitroxyl anion
NO^+	=	Nitrosyl cation
NO•	=	Nitric oxide
NO_2^+	=	Nitronium (nitryl) cation
NO_2•	=	Nitrogen dioxide
NOS	=	Nitric oxide synthase
NOX	=	N-like enzymes
NQO1	=	NAD(P)H quinone oxidoreductase 1
Nrf2/ARE	=	Nuclear factor (erythroid-derived 2)-like 2
nSMase	=	Neutral sphingomyelinase
O_2•$^-$	=	Superoxide
O_2••	=	Oxygen radical
O_3	=	Ozone
OH•	=	Hydroxyl-radical
ONE	=	4-oxo-2-nonenal
ONOO•	=	Peroxynitrite
$ONOOCO_2^-$	=	Nitroso peroxo carboxylate
ONOOH	=	Peroxynitrous acid
OxPC	=	Phosphatidylcholine
P66SHC (SH1)	=	SHC adaptor protein 1
PAI-1	=	Plasminogen activator inhibitor-1
PARK7/DJ-1	=	Parkinson disease protein 7/ protein deglycase DJ-1
PARP	=	Poly (ADP-ribose) polymerase
PC-12	=	Neuronally differentiated pheochromocytoma cells
PD	=	Parkinson's disease
Pin1	=	Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1
PINK1	=	PTEN-induced putative kinase 1
PKB	=	Protein kinase B
PKC	=	Protein kinase C
PP1	=	Protein phosphatase 1
PP2A	=	Protein phosphatase 2A
ppm	=	Parts per million
PPP1R15A	=	protein/ Protein phosphatase 1 regulatory subunit 15A
PQ	=	Paraquat
PUFAs	=	Polyunsaturated fatty acids
RAGE	=	Receptor for Advanced Glycation End Products
RCAN1	=	Regulator of calcineurin 1
RNA	=	Ribonucleic acid
RNS	=	Reactive nitrogen species
RO•	=	Alkoxyl
RO_2•	=	Peroxyl
RONS	=	Reactive oxygen nitrogen species
ROOH	=	Organic peroxides
ROS	=	Reactive oxygen species
ROT	=	Rotenone
SDH	=	Succinate dehydrogenase
–SH	=	Thiol /sulfhydryl groups
SOD	=	Superoxide dismutase
SRXN1	=	Sulfiredoxin 1
SSRIs	=	Selective serotonin reuptake inhibitor
STZ	=	Streptozotocin
TBARS	=	Thiobarbituric acid reacting substance
TGF-β	=	Transforming growth factor beta
THA	=	Tacrine
TNF-α	=	Tumour necrosis factor- a
TSPO	=	Translocator protein

TXNRD1	=	Thioredoxin reductase 1
UCH-L1	=	Ubiquitin carboxy-terminal hydrolase L1
UGT	=	UDP-glucuronosyltransferase
VCAM-1	=	Vascular adhesion cell molecule-1
γ-GCS	=	γ-glutamyl -cysteinyl synthase
σ1Rs	=	Sigma 1 receptor

AUTHOR'S CONTRIBUTIONS

Kyriaki Hatziagapiou: collected literature, drafted the manuscript. Eleni Kakouri: collected literature, drafted the manuscript. Kostas Bethanis: proof-read the manuscript, provided critical review. George I. Lambrou: drafted the manuscript, proof-read the manuscript, provided critical review, gave final permission for submission. Petros A. Tarantilis: gave final permission for submission.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

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