Journal of Integrative Neuroscience

J. Integr. Neurosci. **2024**; 23(3): 53
https://doi.org/10.31083/j.jin2303053

Review

Brain Mitochondria as a Therapeutic Target for Carnosic Acid

Vittoria Infantino[1], Ilaria Pappalardo[1], Anna Santarsiero[1], Swapnil Tripathi[2], Gyanendra Singh[2], Marcos Roberto de Oliveira[3,4,*]

[1]Department of Science, University of Basilicata, 85100 Potenza, Italy
[2]Division of Toxicology, ICMR-National Institute of Occupational Health, 380016 Ahmedabad, India
[3]Department of Biochemistry, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul (UFRGS), CEP 90035-000, Porto Alegre, Rio Grande do Sul, Brazil
[4]Study Group on Neurochemistry and Neurobiology of Bioactive Molecules, Department of Chemistry, Federal University of Mato Grosso (UFMT), CEP 78060-900, Cuiaba, Mato Grosso, Brazil
*Correspondence: mrobioq@gmail.com; deoliveiramr@ufrgs.br (Marcos Roberto de Oliveira)
Academic Editor: Gernot Riedel
Submitted: 21 August 2023 Revised: 8 October 2023 Accepted: 18 October 2023 Published: 7 March 2024

Abstract

Carnosic acid (CA), a diterpene obtained mainly from *Rosmarinus officinalis* and *Salvia officinalis*, exerts antioxidant, anti-inflammatory, and anti-apoptotic effects in mammalian cells. At least in part, those benefits are associated with the ability that CA modulates mitochondrial physiology. CA attenuated bioenergetics collapse and redox impairments in the mitochondria obtained from brain cells exposed to several toxicants in both *in vitro* and *in vivo* experimental models. CA is a potent inducer of the major modulator of the redox biology in animal cells, the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2), which controls the expression of a myriad of genes whose products are involved with cytoprotection in different contexts. Moreover, CA upregulates signaling pathways related to the degradation of damaged mitochondria (mitophagy) and with the synthesis of these organelles (mitochondrial biogenesis). Thus, CA may be considered an agent that induces mitochondrial renewal, depending on the circumstances. In this review, we discuss about the mechanisms of action by which CA promotes mitochondrial protection in brain cells.

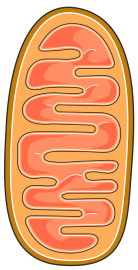
Keywords: carnosic acid; mitochondria; neurons; brain; antioxidant; Nrf2

1. Introduction

Mitochondria are the cytoplasmic organelles of eukaryotic cells historically recognized as the mainly responsible for the production of metabolic energy as they house the oxidative phosphorylation (OXPHOS) [1]. Different metabolic pathways, such as the tricarboxylic acid (TCA) cycle, β-oxidation, urea cycle, gluconeogenesis, and synthesis and degradation of ketone bodies, among others, occur completely or partially in the mitochondria, depending on the tissue [2] (Fig. 1).

Mitochondria represent a significant source of reactive oxygen species (ROS) and are involved in calcium ions (Ca^{2+}) homeostasis modulation [3]. Any disturbance in Na^+, K^+-ATPase function leads to Ca^{2+} overload, which can affect mitochondrial dehydrogenases and cellular energetics, hence cell death [4]. Mitochondria are crucial for storing intracellular Mg^{2+}, that impacts mitochondrial function, in particular energy metabolism, mitochondrial Ca^{2+} handling, and apoptosis [5]. Furthermore, mitochondria play crucial roles in cell signaling, cell death, immune response [6,7], aging processes, and the onset of degenerative disease [8,9].

Mitochondria are double-membrane surrounded organelles: the outer mitochondrial membrane (OMM), freely permeable due to the presence of a large number of porins [10], and the inner mitochondrial membrane (IMM),



- **Tricarboxylic acid cycle (Krebs cycle) reactions**
- **Oxidative phosphorylation system**
- **ATP production**
- **Source of reactive species**
- **Metabolism of Ca^{2+} ions**
- **Gluconeogenesis reactions**
- **Urea cycle**
- **Metabolism of ketone bodies**
- **Metabolism of certain hormones**
- **Stress adaptation**

Fig. 1. A summary of the roles exerted by the mitochondria in mammalian cells. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license. ATP, adenosine triphosphate; Ca^{2+}, calcium ions.

structured in cristae, so highly impermeable that almost all ions and molecules require membrane transporters to cross it [11]. The IMM is the primary site of adenosine triphosphate (ATP) synthesis, and it has a high percentage content of proteins involved in OXPHOS and transport of metabolites between the cytosol and the mitochondria [12]. The double-membrane system demarcates two independent compartments: the intermembrane space and the mitochondrial matrix. The internal matrix contains numerous en-

zymes involved in oxidative metabolism as well as the mitochondrial DNA (mtDNA) [13]. Mitochondria are unique among cytoplasmic organelles since their own maternally inherited, circular, and double-stranded DNA, closely resembles a prokaryotic genome [14]. The mtDNA encodes some proteins involved in the OXPHOS system, ribosomal RNAs and most of the transfer RNAs required for protein translation within mitochondria [15].

The TCA cycle is the central hub of metabolism where molecules derived from different metabolic pathways, among which glycolysis and oxidation of fatty acids, are oxidized and the energy obtained is stored in nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$). The high-energy electrons derived from NADH and $FADH_2$ are transferred to molecular oxygen (O_2) through the respiratory chain, localized in the IMM, and formed by the complexes I (NADH dehydrogenase), II (succinate dehydrogenase), III (ubiquinol-cytochrome c reductase), and IV (cytochrome c oxidase) [16]. The energy arising from the flux of electron is converted to potential energy, stored in an electrochemical gradient across the membrane, and used by the complex V (ATP synthase) to drive ATP synthesis [16]. TCA cycle intermediates serve as substrates for the biosynthesis of various molecules, including heme and macromolecules [17], and act as signaling molecules in epigenetics, immunity, hypoxic response, and cell homeostasis [18–23].

Since O_2 is the final electron acceptor of the respiratory chain, mitochondria are the main oxygen consumers (more than 90% of cell O_2) and, consequently, the main producers of ROS including the radical anion superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), by-product of the respiratory chain [13,24,25]. Manganese superoxide dismutase (Mn-SOD) catalyzes the conversion of $O_2^{\bullet -}$ to H_2O_2, which can further be converted by mitochondrial aconitase to the highly reactive $OH^{\bullet}$ [26]. However, the respiratory chain is not the only one source of ROS in the mitochondria: notably, cytochrome P450 (CYP) enzymes release $O_2^{\bullet -}$ and H_2O_2 as by-products of metabolism of different organic substrates (xenobiotics, steroid hormones and others) [27], and the enzyme monoamine oxidase (MAO) produces H_2O_2in the process of monoamine neurotransmitters degradation [28]. ROS are necessary for cell survival since they participate in cellular signaling processes [29], but the overproduction can cause OXPHOS dysfunction and damage to nucleic acids (DNA mutations), lipids (peroxidation of membrane lipids), proteins (glycation, oxidation, nitration of proteins, and inactivation of enzymes), therefore being associated with the development of cardiovascular, neurodegenerative and metabolic disorders, cancer, inflammation, and aging [30]. Multiple enzymatic and non-enzymatic antioxidant systems scavenge the ROS and limit their toxicity, notably glutathione peroxidase, catalase, glutathione (GSH) and vitamin E [31–33].

Mitochondria regulate different types of cell death, with an essential role in apoptosis [34]. Various cellular stresses, among which DNA damage, hypoxia, viral infection, induce the intrinsic pathway of apoptosis initiated by the opening of the mitochondrial permeability transition pore (mPTP) located in the IMM. The aperture of the mPTP leads to the IMM swelling and the rupture of the OMM, followed by the leakage of cytochrome c, apoptosis-inducing factor (AIF) and endonuclease G [34]. Cytochrome c binds and activates apoptotic protease activating factor (Apaf-1), forming a complex known as apoptosome [35]. Apoptosome binds to and activates procaspase-9 to become the initiator caspase-9, which subsequently cleaves and activates the downstream effector caspases 3, 6, and 7, inducing apoptosis. Caspase-independent nuclear translocation of AIF and endonuclease G triggers chromatin condensation and degradation. Members of the B-cell lymphoma 2 (Bcl-2) family control these events exhibiting pro- and/or anti-apoptotic properties [36]. Mitochondria take part also in other cell death mechanisms [37], among which pyroptosis [38], necroptosis, and ferroptosis [39].

Mitochondrial biogenesis and mitochondria-selective autophagy (mitophagy), which fosters the elimination of damaged mitochondria, are two opposing processes that must be finely regulated and balanced to preserve mitochondrial homeostasis and ensure cellular adaptation to metabolic shifts, stress signals and environmental stimuli, such as aerobic exercise or caloric restriction [40]. Imbalance between mitochondrial biogenesis and mitophagy leads to development of countless pathologic conditions [41].

Mitochondrial biogenesis is the physiological response to adapt the number of mitochondria to energy demands by which new organelles are formed by the growth (increase in mitochondrial mass) and division of preexisting mitochondria [42,43]. Mitochondrial biogenesis is controlled by both nuclear and mitochondrial genomes [43]. The master regulator in mitochondrial biogenesis is peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α), that induces the activation of transcription factors nuclear respiratory factor 1 (NRF1) and GA-binding protein (GABP), also known as nuclear respiratory factor 2 [44,45], leading in turn to the upregulation of mitochondrial transcription factor A (TFAM) as well as other nuclear genes encoded mitochondrial subunits of electron transport chain complexes [46]. TFAM translocates to the mitochondria, where it stimulates the replication and transcription of mtDNA [47,48]. TFAM is also involved in mtDNA repair [49]. PGC-1α is deacetylated by nicotinamide adenine dinucleotide (NAD^+)-dependent deacetylase sirtuin 1 (SIRT1), when cellular NAD^+ levels increase in an adenosine monophosphate (AMP)-activated protein kinase (AMPK)-dependent manner [50]. Thus, the modulation of the axes AMPK/SIRT1/PGC-1α represents a pharmacological target to modulate the mitochondrial biogene-

sis [50]. Impaired mitochondrial biogenesis contributes in pathogenesis of cardiovascular [51] and neurodegenerative diseases, including Alzheimer's disease (AD) [52], Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) [8,9].

Mitophagy is the process of selective degradation of damaged and dysfunctional mitochondria through autophagy [53]. Mitochondria are enclosed by a membrane, forming autophagosomes which fuse with lysosomes for hydrolytic degradation. Phosphatase and tensin homolog (PTEN)-induced putative protein kinase 1 (PINK1)/Parkin pathway is the main molecular mechanisms regulating mitophagy [54]. PINK1 is a serine/threonine kinase usually present in low levels. When any damage occurs to mitochondria like mutations in mtDNA, accumulation of misfolded proteins, increased ROS, or depolarization, PINK1 accumulates at the OMM. PINK1 phosphorylates ubiquitin on Ser65 and recruits Parkin from the cytosol, phosphorylating and activating it. Parkin is an E3-ubiquitin ligase that, when activated, triggers the ubiquitination of a wide range of OMM proteins. These events result in the recruitment of autophagy receptors, such as p62/sequestosome 1 (SQSTM1), that facilitate the sequestering of damaged mitochondria within autophagosomes [55]. SQSTM1 comprises a domain interacting with polyubiquitinated proteins on the mitochondrial surface and a motif binding to microtubule-associated protein 1A/1B-light chain 3-II (LC3-II) present on autophagosomes [56]. Mitophagy can occur independently from functional PINK1, allowing ubiquitin-independent recognition of damaged mitochondria. In mammals the known receptors for receptor-mediated mitophagy include Bcl-2 interacting protein 3 (BNIP3), its analog NIX (BNIP3L) and FUN14 domain-containing protein 1 (FUNDC1) [54,57]. These adapters detect mitochondrial damage and guide dysfunctional mitochondria to the autophagosome by changing their subcellular location or the proteins they interact with. FUNDC1 is a mitochondrial protein located in the OMM protein sensitive to hypoxia. The affinity of FUNDC1 for LC3 is regulated by phosphorylation at Ser17 and Ser13 under different stresses. While phosphorylation at Ser17 activates mitophagy, phosphorylation at Ser13 inhibits this process [55,58,59]. Mitophagy declines with aging determining accumulation of defective mitochondria, increased oxidative stress, and higher cell apoptosis [60].

In keeping with the multiple functions, mitochondria can change rapidly both form (size, shape, and position) and number to meet the physiological needs of cells undergoing the two opposing processes of fission and fusion [61,62]. Mitochondrial homeostasis is strictly dependent on the equilibrium between fusion and fission dynamics which are important for mitochondrial inheritance and maintenance of functions (e.g., efficient OXPHOS, transport and regulation of mitophagy). Fission involves the separation of both OMM and IMM and their subsequent rejoining in the correct orientation, besides the properly partitioning of mitochondrial proteins and mtDNA so that each daughter organelle can function normally. In physiological condition, fission happens prior to cell division to guarantee that organelles are equally distributed between daughter cells [62]. Fission is also required as a preliminary event to mitophagy, to allow for segregation of damaged organelles, their inclusion in autophagosomes and finally their degradation [63,64]. Mitochondrial dynamics require specialized nuclear-encoded protein, mainly represented by GTP-hydrolyzing proteins (GTPases) belonging to the dynamin superfamily. Three central players are (1) mitofusin 1 and mitofusin 2 (OMM fusion) [65], (2) optic atrophy 1 (OPA1) (IMM fusion) [66], and (3) dynamin-related protein 1 (Drp1) (division of IMM and OMM) [67]. Perturbations in mitochondrial dynamics have detrimental consequences on bioenergetic supply and represent a common feature of different neurogenerative disorders such as AD, PD, and HD [9].

1.1 Alterations in Mitochondrial Physiology in Neurodegenerative Diseases

AD usually affects people over the age of 65 and accounts for at least two-thirds of dementia cases [68]. AD is characterized by the accumulation of beta-amyloid (Aβ) plaques and phosphorylated Tau (pTau) protein in the brain [69]. Aβ and pTau alter mitochondrial integrity and worsen mitochondrial dysfunction, resulting in increased oxidative stress which in turn promotes the accumulation of these two proteins [70]. Aβ and pTau-induced oxidative damage inhibits mitophagy [71]. Moreover, Aβ and pTau determine the enlargement of mPTP causing the decrease of the intermembrane potential [72], the leakage of cytochrome C and, thus, the triggering of apoptosis [73]. Both mitochondrial dynamics and biogenesis are impaired in AD where decreased expression of PGC-1α, TFAM and GABP proteins are observed [74,75]. Deficiency in complex IV and citrate synthase activities have also been found in AD patients [76,77]. Similarly, mitochondria from PD patients present impaired architecture, function (for example, complex I dysfunction), dynamics (disruption of mitochondrial fusion [78,79]) mitochondrial biogenesis, mitophagy, increased ROS production, and Ca^{2+} imbalance [80,81]. Mitochondria from patients suffering from HD show decreased activity of complex I [82] and IV [83], and impaired TCA function [84]. Beyond metabolic alterations, mitochondria show abnormal fission [85], reduced mitochondrial biogenesis [86] and inefficient mitophagy [87].

Many studies have been carried out to develop therapeutic strategies with the aim of attenuating mitochondrial dysfunction and restoring mitochondrial homeostasis in neurodegenerative diseases. Approaches target various mitochondrial processes such as energy metabolism, ROS generation, mitochondrial dynamics, mitochondrial biogenesis and mitochondrial protein synthesis. Different

plant-derived natural products have been shown to be effective in delaying or treating neurodegenerative diseases via modulation of mitochondrial dysfunction [88]. Increasing evidence is establishing the relevance of carnosic acid (CA) as a promising neuroprotective agent.

1.2 Carnosic Acid

Carnosic acid (CA; $C_{20}H_{28}O_4$), commonly known as salvin, is a plastidial phenolic diterpene found in plant species of the Lamiaceae family with antioxidant properties [89]. Structurally, it is characterized by an 8,11,13-triene abietane skeleton replaced by hydroxy groups at positions 11 and 12 and a carboxyl group at position 20 (Fig. 2) [90]. This polyphenolic carbotricyclic compound is a monocarboxylic acid and a conjugate acid of the carnosate (Information. PubChem Compound Summary for CID 65126, Carnosic Acid. [(accessed on 9 July 2023)]; Available online: https://pubchem.ncbi.nlm.nih.gov/compound/Carnosic-acid).

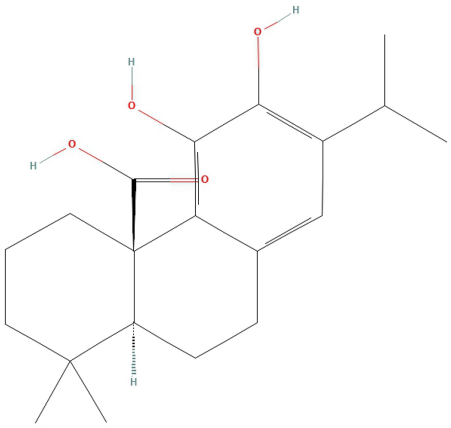


Fig. 2. Carnosic acid chemical structure. The chemical structure of carnosic acid was obtained from PubChem.

Also *in planta*, this molecule can give rise to various dehydrogenation derivatives such as carnosol, rosmanol and isorosmanol. If plants are subjected to high drought conditions or intense solar radiation, CA can be converted to methyl derivatives. It is clear that this molecule exerts a dual protective role in the rosemary plant against environmental stresses by capturing free radicals in the chloroplasts and preventing the breakdown of biological membranes [91–93]. As far as we know, CA has been identified in only one plant family, in only one out of 10 subfamilies of Lamiaceae (Nepetoideae), in only two out of 66 tribes (Mentheae, Ocimum), and in only nine out of 220 genera [89]. Most probably such a poor distribution in the plant kingdom is due to a very specific evolutionary strategy, but also to a scarcity of research, as many plant species still remain unknown and/or unstudied [89]. CA is not evenly diffused in plants, being present more in the aerial parts [94,95] and its levels also depend on the phase of development. The biosynthesis and accumulation of CA occurs solely in young rosemary leaves at the branch apexes, where the diterpene molecule is partly employed during leaf formation and ageing [92,96–98].

Bioavailability studies are few. It was reported that, in rats, 6 hours after oral administration (64.3 $\pm$ 5.8 mg/kg), the bioavailability of CA was 40.1%, and its excretion in feces was 15.6 $\pm$ 8.2% after 24 hours. When administered orally, CA is absorbed into the bloodstream and traces were detected in the intestine, liver and muscle tissue of the abdomen and legs in rats [99]. In another study, Romo Vaquero *et al.* [100] detected CA and its derivatives 25 minutes after oral administration in Zucker rats of a CA-enriched rosemary extract, mainly in the intestine, liver and plasma. Some amounts of CA and its metabolites were also found in the brain, resulting in beneficial effects for this complex organ. In another study [101], the acute and 30-day oral toxicity of CA was evaluated in Wistar rats and the acute oral lethal dose (LD_{50}) in the range of 7100 mg/kg body weight was identified. A high dose of CA induced a slight decrease in body weight gain in the chronically treated rats liken to the control group. In addition, only slight pathological alterations were observed in heart, liver, and kidney. These considerations indicate a low toxicity profile of the molecule.

Research over the past decade has revealed numerous bioactive abilities of CA including antioxidant, anti-inflammatory and anti-cancer activities, among others (Table 1, Ref. [92,100–136]; Fig. 3). This fat-soluble compound is known for its documented antioxidant properties, which have enabled it to find various industrial applications in food and beverages, personal care, nutrition and health [89]. Most likely, the antioxidant properties of CA are linked to the presence of a catechol moiety and have been studied mainly *in vitro* in a wide variety of artificial and/or model systems [93,102]. CA has been shown to protect fatty acids and triglycerides from oxidation in bulk and emulsified lipid systems [103–105]. Pearson *et al.* [106] showed how CA prevents oxidation of low-density lipoproteins in human aortic endothelial cells. Wijeratne and Cuppett [107] observed CA-mediated lowering of oxidative stress occurring by lipid hydroperoxide in Caco-2 cells. Moreover, it has been reported that CA reduces lipid peroxidation in rat liver microsomes and brain [100,108]. Foods such as raw or cooked meat and oil have been protected from oxidation by CA, generally with greater efficiency than synthetic antioxidants [109–112]. CA has been shown to have a very high reactivity towards ROS, being quickly oxidized and converted to a variety of metabolites in the process. Acting as a scavenger of ROS, CA eliminates both singlet oxygen, an excited form of oxygen, and free radicals. Furthermore, oxidized CA derivatives were found in rosemary leaves under both control and stress conditions, and prolonged exposure to darkness resulted in a

Table 1. Bioactive properties of CA.

Bioactive properties	Mechanism of action	References
Antioxidant	- Protection of fatty acids and triglycerides from oxidation	[103–107]
	- Inhibition of lipid peroxidation	[100,108–112]
	- H_2O_2 elimination Elimination of singlet oxygen and free radicals	[92]
Antimicrobial	- Antilisterial, antibacterial effect against Gram-positive and Gram-negative bacteria	[113–120]
	- Modulation of the bacterial membrane permeability	[121–123]
Anti-cancer	- Proapoptotic effect	[124]
	- Antiproliferative activity	[125]
	- Anti-angiogenic action	[126]
	- Chemoprotective function	[127]
	- Antiplatelet properties	[128]
Anti-inflammatory	- Interleukin suppression	[129]
	- Blocking of proto-oncogene tyrosine-protein kinase Src pathway	
	- Increasing of PGE_2 level	[130]
	- Inhibition of NO release	[102]
	- TNF-α reduction by activation of TACE	[131]
	- NF-κB/p65/p50 inhibition	[132]
	- PPARγ cleavage suppression	[133,134]
Anti-adipogenic	- Fasting glycaemia and plasma cholesterol levels reduction	[135,136]
	- Inhibition of pancreatic lipase activity	
	- PPARγ protein expression and activity reduction	
	- Hepatocyte lipid accumulation reduction	
	- Reduction of inflammatory cytokines expression (IL-1β, IL-12, IL-17, IFN-γ, MCP-1 and MIP-1β)	[101]
	- Increasing of ATP, acetyl CoA, NAD(P)(+), and NAD(P)H	
	- EGFR, MAPK, AMPK, and ACC activation	
	- Inhibition of palmitate-induced cellular lipid accumulation	
	- Stimulation of EGFR and MAPK phosphorylation	

CA, carnosic acid; H_2O_2, hydrogen peroxide; PGE_2, prostaglandin E_2; NO, nitric oxide; TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor-κB; PPARγ, peroxisome proliferator-activated receptor-γ; AMPK, adenosine monophosphate (AMP)-activated protein kinase; IL, interleukin; IFN-γ, interferon-γ; MCP-1, monocyte chemoattractant protein-1; MIP-1β, macrophage inflammatory protein-1β; CoA, coenzyme A; NAD(P)(+), nicotinamide adenine dinucleotide phosphate, oxidized form; NAD(P)H, nicotinamide adenine dinucleotide phosphate, reduced form; ACC, acetyl-CoA carboxylase; EGFR, epidermal growth factor receptor; MAPK, mitogen-activated protein kinase; TACE, tumor necrosis factor α-converting enzyme.

striking reduction in their concentrations. These data indicate the chronic oxidation of CA in plants by light and suggest a protective role of CA even under low light conditions [92].

Antimicrobial activities have been described for CA [93,102,113,114]. This property was determined by evaluating the antilisterial effect of a series of rosemary phenolic compounds and it was found that CA exhibited the strongest antilisterial activity [115] when tested for 24 h at 30 °C after inoculation. Moreover, this activity was further enhanced at low pH and high NaCl content. Other Gram-positive bacteria, such as *Bacillus*, *Enterococcus*, *Streptococcus* and *Staphylococcus*, and Gram-negative bacteria, such as *Escherichia*, *Salmonella* and *Campylobacter*, were also sensitive to CA [113,116–120]. The proposed antimicrobial action mechanism provides that, thanks to its lipophilic structure, CA can be inserted into the bacterial membrane [123], where the hydrogen binding donor group could interact with phosphorylated membrane groups [121] or that CA modules the release of ethyl bromide responsible for membrane permeability [122].

Anti-cancer effects of CA have also been reported due to its proapoptotic [124], antiproliferative [125], anti-angiogenic [126], chemoprotective [127], antitumor [137, 138] and antiplatelet properties [128]. Some *in vitro* studies have highlighted the preventive [139,140] and inhibitory [102,135,141,142] action against cancer of plant extracts containing CA.

Numerous other investigations have shown the anti-inflammatory properties of plant extracts rich in CA. These effects include interleukin suppression, blocking of the proto-oncogene tyrosine-protein kinase Src pathway [129], regulation of prostaglandin E_2 (PGE_2) secretion [130], inhibition of nitric oxide ($NO^{\bullet}$) release [102],

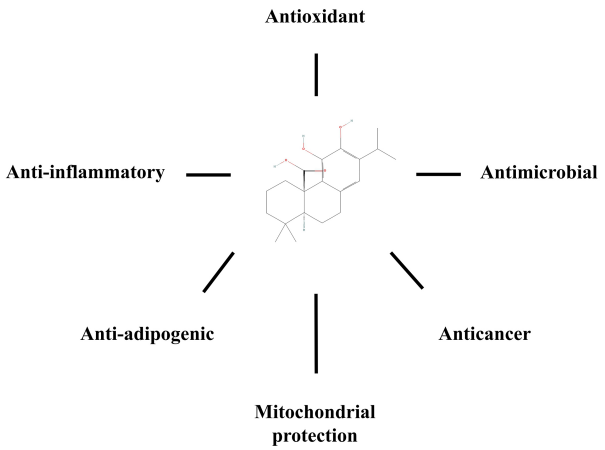


Fig. 3. A summary of the biological effects promoted by carnosic acid in mammals. The chemical structure of carnosic acid was obtained from PubChem.

reduction of tumor necrosis factor-α (TNF-α) by activation of tumor necrosis factor α-converting enzyme (TACE) [131,133], inhibition of the transcription factor nuclear factor-κB (NF-κB) [132] and suppression of peroxisome proliferator-activated receptor-γ (PPARγ) cleavage [134]. Anti-adipogenic properties are reported for CA. Indeed, CA administration resulted in significant weight loss and improved glycemic response [135,136,143].

Furthermore, CA shows the capacity to promote mitochondrial protection in neural cells, so it could be used as a therapeutic agent for the treatment of neurodegenerative disorders such as AD, PD and HD, in which mitochondrial dysfunction plays a central role in initiation and development [144]. This review summarizes the *in vitro* and *in vivo* studies performed so far concerning the effect of CA on mitochondrial function in neurodegenerative diseases.

2. *In Vitro* Experimental Models

CA exerts cytoprotection in several *in vitro* experimental models involving brain cells [89,144,145]. CA induces antioxidant effects mainly by a mechanism associated with the upregulation of both non-enzymatic and enzymatic antioxidant defenses in neurons [144] (Fig. 4). Satoh *et al*. [146] reported that CA at 10 µM for 6–48 h stimulated the antioxidant-responsive element (ARE)/nuclear factor erythroid 2-related factor 2 (Nrf2), upregulating the enzymes heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase-1 (NQO-1), and also the catalytic (GCLC) and modifier (GCLM) subunits of γ-glutamate-cysteine ligase (γ-GCL, a major enzyme involved in the production of GSH, the major non-enzymatic antioxidant in animal cells) in the rat pheochromocytoma subclone PC12h cells. Moreover, Kosaka *et al*. [147] observed that CA promoted neurite outgrowth in the PC12h cells by a mechanism dependent on Nrf2, suggesting that this diterpene would be able to modulate neuronal differentiation and plasticity. CA at 10 µM also upregulated GSH production in the mouse hippocampal HT22 cell line exposed to glutamate [148], suggesting that the effects promoted by CA are not limited to just one cell type. Similarly, Chen *et al*. [149] reported that CA at 1 µM for 6–24 h stimulated the production of GSH and upregulated the GCLC and GCLM subunits in the human dopaminergic SH-SY5Y cells. The authors also observed that CA stimulated the translocation of Nrf2 to the cell nucleus, enhancing the activity of ARE. Moreover, CA suppressed the mitochondria-related apoptosis triggered by 6-hydroxydopamine (6-OHDA) by suppressing caspase-3 activation and poly (ADP-ribose) polymerase (PARP) cleavage in SH-SY5Y cells. Interestingly, blockade of the synthesis of GSH by using L-buthionine sulfoximine (BSO) abrogated the anti-apoptotic effects induced by CA in that experimental model. Fig. 4 summarizes these important findings related to the metabolism of GSH in CA-treated cells. In agreement with these data, Lin *et al*. [150] showed that CA at 1 µM for 18 h stimulated the phosphatidylinositol 3-kinase (PI3K)/Akt/NF-κB signaling pathway and upregulated the Pi form of glutathione-*S*-transferase (GSTP) in the SH-SY5Y cells. Silencing of GSTP abrogated the mitochondria-related anti-apoptotic effect (i.e., suppression of caspase-3 activation) caused by CA in the 6-OHDA-challenged SH-SY5Y cells. GSTP depends on GSH to mediate phase II detoxification reactions [151]. Thus, it seems that the CA-induced upregulation of the production of GSH prevents cells collapse by a mechanism that does not depend only on the antioxidant role of that tripeptide. The same research group reported that CA at 1 µM attenuated the 6-OHDA-induced increase in the immunocontent of Bcl-2-associated X protein (Bax) (which mediates apoptosis by interacting with the mitochondria) and the decrease in the levels of Bcl-2 (which blocks apoptosis after interacting with the mitochondria) in the SH-SY5Y cells [152]. Bax activator molecule 7 (BAM7), an activator of Bax, abrogated the CA-induced anti-apoptotic effects in the 6-OHDA-treated SH-SY5Y cells, indicating that the mitochondria are central in the mechanism by which CA promotes anti-apoptotic actions.

Even though the existence of findings indicating that CA would be capable to promote neuroprotective actions, it was not demonstrated whether those effects would be linked to the ability of this diterpene in modulating mitochondrial physiology (which involves bioenergetics, redox biology, dynamics, and biogenesis of the organelles). Furthermore, it was not clearly demonstrated whether Nrf2 (or its downstream targets) would present a role in the mechanism promoted by CA with regard to the mitochondria. Thus, it was investigated whether CA would prevent mitochondrial dysfunction in different *in vitro* experimental designs related to neurotoxicity. We found that CA at 1 µM for 12 h attenuated the mitochondrial dysfunction induced by the agrochemical paraquat (a mitochondrial toxicant) on the human dopaminergic SH-SY5Y cells [153]. CA blocked the Bax

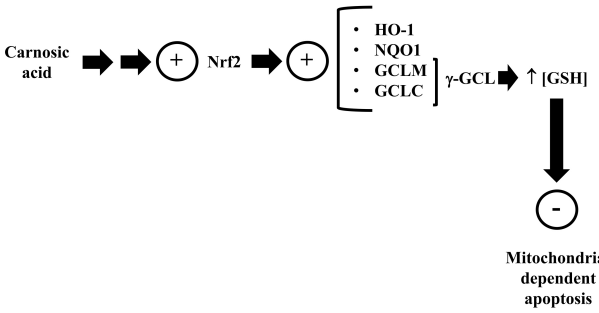


Fig. 4. Carnosic acid stimulates the transcription factor Nrf2, leading to an upregulated expression and synthesis of several cytoprotective enzymes, including those involved with the metabolism of GSH. Please, read the text for detailed information. HO-1, heme oxygenase-1; NQO1, NAD(P)H quinone oxidoreductase-1; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modifier subunit; γ-GCL, γ-glutamate-cysteine ligase; Nrf2, nuclear factor erythroid 2-related factor 2; GSH, glutathione.

upregulation induced by paraquat, consequently decreasing the release of cytochrome c from the mitochondria to the cytosol. Moreover, CA attenuated the paraquat-induced upregulation in the activity of the pro-apoptotic caspases-9 and -3, resulting in a decrease in the fragmentation of DNA. CA significantly reduced the impact caused by paraquat on the activity of the Complexes I and V, as well as on the mitochondrial membrane potential (MMP) and production of ATP, indicating a role for CA in rescuing mitochondrial function (Fig. 5). In the same work, we demonstrated that CA prevented the paraquat-induced increase in the mitochondrial levels of malondialdehyde (an index of lipid peroxidation) and protein carbonylation. These effects may be linked to the ability of CA in upregulating the levels of GSH and Mn-SOD in the mitochondria of the SH-SY5Y cells (Fig. 6). Nonetheless, it was not examined in that work. It was found that blockade of PI3K/Akt or knocking down of the transcription factor Nrf2 suppressed the mitochondria-related cytoprotection caused by CA in that experimental model, suggesting that the PI3K/Akt/Nrf2 axis would be involved in mediating the effects promoted by CA in the SH-SY5Y cells. Similarly, de Oliveira *et al.* [154] reported that blockade of the PI3K/Akt/Nrf2 axis attenuated the effects caused by CA on the mitochondria of the SH-SY5Y cells challenged with methylglyoxal, a dicarbonyl commonly associated with diabetes *mellitus* and neurodegeneration [155] (Fig. 5).

CA is a potent activator of Nrf2, leading to the expression of several enzymes associated with cytoprotection in different cell types [146,149]. The enzyme HO-1, one of the most studied downstream targets of Nrf2, mediates heme degradation generating biliverdin, carbon monoxide (CO), and free iron and promoting cytoprotection, mainly

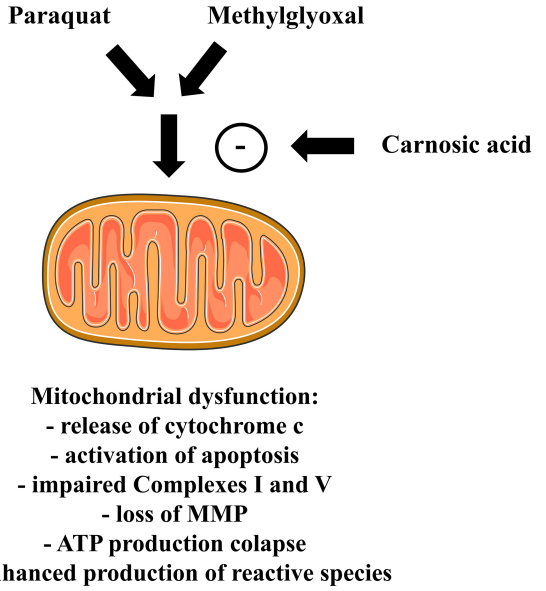


Fig. 5. Carnosic acid attenuates the effects caused by either paraquat or methylglyoxal on the mitochondria of cultured cells. Please, read the text for detailed information. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license. MMP, mitochondrial membrane potential.

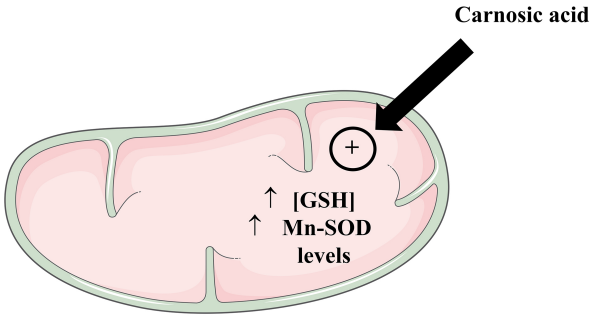


Fig. 6. Carnosic acid upregulates the levels of GSH and Mn-SOD in the mitochondria of cultured cells. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license. Mn-SOD, manganese-dependent superoxide dismutase.

by antioxidant and anti-inflammatory-related mechanisms, in different human cell types [156]. Biliverdin generated by HO-1 is further converted into bilirubin by the enzyme biliverdin reductase (BVR) [157]. Since it was showed that CA upregulated HO-1 in previous works [146,153], we investigated whether this enzyme would exert a role in mediating the protection of mitochondria in CA-treated SH-SY5Y cells exposed to paraquat [158]. It was found that the inhibition of HO-1 by using zinc protoporphyrin-IX (ZnPP-IX) blocked the preventive effects caused by CA on the mi-

Table 2. A summary of the effects induced by CA on the brain mitochondria in *in vitro* experimental models.

Cell line	Experimental model	Mitochondria-related main effects	Interpretation	References
SH-SY5Y cells	CA at 1 μM for 12 h before exposure of the cells to 6-hydroxydopamine (6-OHDA)	- CA upregulated ARE/Nrf2, GCLC), GCLM, and GSH - CA prevented the 6-OHDA-dependent activation of caspase-3 and the cleavage of PARP - L-buthionine sulfoximine (BSO) at 100 μM blocked the mitochondria-related anti-apoptotic effects induced by CA	CA depends on GSH to suppress the mitochondria-related pro-apoptotic effect triggered by 6-OHDA	[149]
SH-SY5Y cells	CA at 1 μM for 18 h before the administration of 6-OHDA for 12 or 18 h	- CA upregulated the PI3K/Akt/NF-κB axis - CA increased the immunocontent and activity of the enzyme GSTP - CA blocked the activation of caspase-3 by a mechanism dependent on the PI3K/Akt/GSTP axis	CA depends on the PI3K/Akt/NF-κB/GSTP axis to block the mitochondria-related pro-apoptotic effect induced by 6-OHDA	[150]
SH-SY5Y cells	CA at 1 μM for 8 h prior to the exposure of the cells to 6-OHDA for additional 18 h	- CA prevented the 6-OHDA-induced downregulation of GCLC, GCLM, glutathione reductase (GR), and superoxide dismutase (SOD) - A pretreatment with CA blocked the 6-OHDA-induced alterations on the levels of Bax, Bcl-2, caspase-3, and cleaved PARP - BAM7, an activator of Bax, suppressed the mitochondria-related anti-apoptotic effects elicited by CA	The blockade of the mitochondria-related pro-apoptotic signal is an important part of the anti-apoptotic action induced by CA	[152]
SH-SY5Y cells	CA at 1 μM for 12 h prior to the exposure of the cells to paraquat at 100 μM for different periods	- The pretreatment with CA attenuated the paraquat-induced increase in the levels of Bax, the release of cytochrome c to the cytosol and the activation of the caspases-9 and -3 - CA promoted mitochondrial protection by attenuating the effects of paraquat on the activity of the Complexes I and V and on the levels of ATP and MMP - CA prevented the paraquat-induced oxidation of lipids and proteins in the mitochondria - CA induced an increase in the levels of mitochondrial GSH and Mn-SOD - Inhibition of the PI3K/Akt/Nrf2 signaling pathway suppressed the mitochondrial and cellular protection promoted by CA in the paraquat-treated cells	CA depended on the PI3K/Akt/Nrf2 axis to promote mitochondrial protection in the cells exposed to paraquat (a mitochondrial toxicant)	[153]
SH-SY5Y cells	CA at 1 μM for 12 h before the administration of methylglyoxal at 500 μM for different periods	- CA upregulated Mn-SOD - CA prevented the methylglyoxal-induced decline in the levels of ATP - Inhibition of the PI3K/Akt/Nrf2 signaling pathway abrogated the mitochondria-related protection induced by CA	CA depended on the PI3K/Akt/Nrf2 axis to prevent the mitochondrial and cellular collapses caused by methylglyoxal	[154]
SH-SY5Y cells	CA at 1 μM for 12 h prior to the exposure of the cells to paraquat at 100 μM for different periods	- CA prevented the paraquat-induced loss of MMP and decline in the levels of ATP - CA prevented the redox impairment (lipid peroxidation and protein carbonylation and nitration) induced by paraquat on the membranes of mitochondria	CA depended on the Nrf2/HO-1 axis to promote mitochondrial protection in the cells exposed to paraquat	[158]

Table 2. Continued.

Cell line	Experimental model	Mitochondria-related main effects	Interpretation	References
		- The pretreatment with CA attenuated the paraquat-induced decrease in the activity of the Complexes I and V - CA prevented the mitochondria-related pro-apoptotic action triggered by paraquat - Silencing of Nrf2 or inhibition of HO-1 suppressed the mitochondrial and cellular protection induced by CA		
SH-SY5Y cells	CA at 1 μM for 12 h prior to the exposure of the cells to methylglyoxal at 500 μM for different periods	- BSO suppressed the antioxidant effects elicited by CA in the membranes of mitochondria isolated from the cells exposed to methylglyoxal - CA failed to prevent the mitochondrial dysfunction caused by methylglyoxal in the cells exposed to BSO - BSO abrogated the effect caused by CA on the production of the radical anion superoxide ($O_2^{-\bullet}$) by the mitochondria obtained from the methylglyoxal-treated cells	CA relies on GSH to prevent mitochondrial collapse caused by methylglyoxal	[159]
SH-SY5Y cells	CA at 1 μM for 8 h before the administration of 6-OHDA at 100 μM for further 18 h	- CA prevented the 6-OHDA-induced inhibition of the chymotrypsin-like proteasome activity - CA upregulated PINK1 and parkin - CA prevented the 6-OHDA-induced downregulation in PINK1 and parkin - Silencing of parkin or administration of MG132 (an inhibitor of the proteasome) suppressed the mitochondria-related anti-apoptotic effects induced by CA in the cells exposed to 6-OHDA	CA stimulated the PINK1/parkin axis leading to a mitochondria-related anti-apoptotic action in the cells exposed to 6-OHDA	[160]
SH-SY5Y cells	CA at 1 μM for 18 h before the administration of 6-OHDA at 100 μM for additional 12 h	- CA prevented the 6-OHDA-induced decrease in the levels of VDAC1 - CA prevented the 6-OHDA reduction in the levels of ubiquitinated VDAC1 - Silencing of PINK1 suppressed the CA-induced increase in the levels of parkin, VDAC1, and LC3-II - Silencing of PINK1 abrogated the CA-induced decrease in the activation of caspase-3 and cleavage of PARP	CA induced a mitochondria-related anti-apoptotic action by a mechanism dependent on the PINK1/parkin/mitophagy axis	[161]
SH-SY5Y cells	CA at 1 μM was administrated to the cells for 18 h prior to the challenge with 100 μM 6-OHDA for further 9 h	- CA upregulated Mic60 and citrate synthase - Silencing of Mic60 suppressed the CA-induced blockade on the release of cytochrome c - CA upregulated PINK1 - Silencing of PINK1 abrogated the CA-induced effect on the phosphorylation of Mic60 - CA downregulated PKA - CA promoted mitophagy	CA treatment favored the interaction between Mic60 and PINK1 leading to neuronal survival by a mechanism dependent on mitophagy	[162]

Table 2. Continued.

Cell line	Experimental model	Mitochondria-related main effects	Interpretation	References
SH-SY5Y cells	CA at 1 μM for 18 h before the administration of 6-OHDA at 100 μM for further 12 h	- CA upregulated PI3K p100, Beclin1, Atg7, and the conversion of LC3-I into LC3-II - CA stimulated the phosphorylation of mTOR - CA upregulated parkin - Silencing of parkin abrogated the effects of CA on PI3K p100, Beclin1, and Atg7, as well as on the conversion of LC3-I into LC3-II - Inhibition of autophagy suppressed the mitochondria-related antiapoptotic effects caused by CA in the cells challenged with 6-OHDA	CA depended on the induction of autophagy to block the 6-OHDA-elicited mitochondria-related apoptosis in the cells exposed to 6-OHDA	[163]
SH-SY5Y cells	CA at 1 μM for 18 h before the administration of 6-OHDA at 100 μM for further 12 h	- CA prevented 6-OHDA-induced upregulation of ARTS and downregulation of XIAP - CA attenuated the activation of the caspases-9 and -7 - Silencing of parkin suppressed the CA-induced ARTS downregulation and XIAP upregulation in the 6-OHDA-treated cells - Silencing of XIAP abrogated the Ca-induced attenuation of the activation of the caspases-9, -7, and -3 and the cleavage of PARP	CA modulated the parkin/ARTS/XIAP signaling to prevent the 6-OHDA-induced apoptosis in the SH-SY5Y cells	[164]
SH-SY5Y cells	CA at 1 μM for 24 h before the administration of 6-OHDA at 100 μM for further 12 or 18 h	- CA upregulated parkin - CA stimulated the translocation of p65 to the cell nucleus - CA upregulated OPA1 - Silencing of parkin suppressed the effects of CA on p65 and OPA1 - Silencing of OPA1 abrogated the anti-apoptotic effects caused by 6-OHDA in the cells	CA depended on the parkin/IKK/NF-κB/OPA1 signaling pathway to suppress the mitochondria-related pro-apoptotic action induced by 6-OHDA in the SH-SY5Y cells	[165]
SH-SY5Y cells	CA at 1 μM for 18 h prior to the administration of 6-OHDA for additional 6–12 h	- CA prevented the 6-OHDA-induced downregulation of NRF1 and TFAM - CA upregulated PGC-1α - CA prevented the 6-OHDA-induced PARIS upregulation - Silencing of parkin suppressed the effects induced by CA on PARIS and PGC-1α - Silencing of PGC-1α abrogated the CA-induced effects on NRF1 and TFAM - Silencing of PGC-1α blocked the mitochondria-related anti-apoptotic effect induced by CA	CA depended on the parkin/PARIS/PGC-1α signaling pathway to stimulate NRF1 and TFAM in cells exposed to 6-OHDA	[166]

ARE, antioxidant-responsive element; PARP, poly (ADP-ribose) polymerase; PI3K, phosphatidylinositol 3-kinase; GSTP, glutathione-S-transferase; PINK1, PTEN-induced putative protein kinase 1; PKA, protein kinase A; XIAP, X-liked inhibitor of apoptosis protein; ARTS, apoptosis-related protein in the TGF-β signaling pathway; OPA1, optic atrophy 1; IKK, IκB kinase; NRF1, nuclear respiratory factor 1; TFAM, transcription factor A; PGC-1α, proliferator-activated receptor-gamma coactivator-1 alpha; PARIS, parkin-interacting substrate; Bcl-2, B-cell lymphoma 2; Bax, Bcl-2-associated X protein; BAM7, Bax activator molecule 7; PTEN, phosphatase and tensin homolog; mTOR, mammalian target of rapamycin; TGF-β, transforming growth factor-β; VDAC1, voltage-dependent anion channel 1; LC3-II, microtubule-associated protein 1A/1B-light chain 3-II.

tochondrial function and redox parameters (please, see Table 2 (Ref. [149,150,152–154,158–166]) for detailed information). Similarly, inhibition of HO-1 abrogated the effects stimulated by CA in SH-SY5Y cells challenged with glutamate [167]. The authors also found that a treatment with either bilirubin or carbon monoxide-releasing molecule-2 (CORM-2, which generates CO) alleviated the alterations caused by glutamate in the mitochondria, suggesting a link between the products generated by the HO-1/BVR axis in mediating the protection of the organelles (Fig. 7).

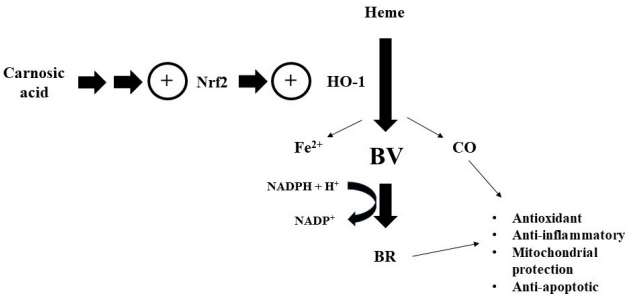


Fig. 7. Carnosic acid upregulates the enzyme HO-1 by a mechanism dependent on the transcription factor Nrf2, causing several beneficial effects related to the mitochondria in cultured cells. Please, read the text for detailed information. CO, carbon monoxide; BV, biliverdin; BR, bilirubin; NADPH, reduced nicotinamide-adenine dinucleotide phosphate; NADP, nicotinamide-adenine dinucleotide phosphate (oxidized form).

Activation of Nrf2 by CA also can lead to increased production of GSH, as previously reported by different research groups [146,148,149]. Thus, we investigated whether the CA-induced upregulation in the Nrf2/GSH axis would be associated with the benefits caused by CA in the mitochondria of SH-SY5Y cells exposed to methylglyoxal, whose biotransformation depends on the availability of GSH [155]. It was found that GSH production blockade, by using BSO, suppressed the actions promoted by CA on the mitochondria of the methylglyoxal-treated cells [159]. CA failed to prevent the redox impairment in the membranes of mitochondria extracted from the methylglyoxal-treated cells exposed to BSO. BSO also suppressed the CA-stimulated effects on the function of mitochondria (which was assessed through the quantification of the activity of the Complexes I and V, of MMP and of the levels of ATP) in the cells challenged with methylglyoxal. Using a different approach, Tamaki *et al.* [148] showed that CA activated the metabolism of GSH in the HT22 cells, leading to cytoprotection against glutamate. Similarly, Nishimoto *et al.* [168] reported that upregulation of GSH attenuated the pro-apoptotic stimuli caused by methylglyoxal in the SH-SY5Y cells. Since GSH also exerts signaling functions in mammalian cells [169], it should be further examined whether other mechanisms, in addition to those related to antioxidant and detoxifying defenses, would be associated with the mitochondria-related benefits promoted by GSH in brain cells (Fig. 8).

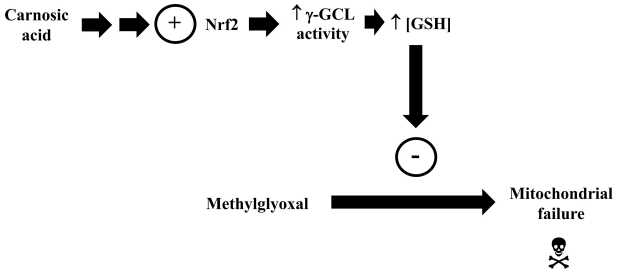


Fig. 8. Carnosic acid stimulates the transcription factor Nrf2, causing an increase in the activity of the enzyme γ-GCL and, consequently, in the levels of GSH. These effects attenuated the mitochondrial failure promoted by methylglyoxal in cultured cells. Please, read the text for detailed information.

In addition to promoting antioxidant and detoxifying defenses that lead to mitochondrial protection, CA also affords cytoprotection by modulating other aspects related to mitochondrial physiology. Lin *et al.* [160] published that CA prevented the pro-apoptotic effects caused by 6-OHDA in SH-SY5Y cells by a mechanism involving upregulation of PINK1 and parkin. The authors also found that silencing of parkin suppressed the mitochondria-related anti-apoptotic effects caused by CA in the 6-OHDA-challegned cells. PINK1, a serine/threonine kinase associated with the IMM, is responsible for recruiting parkin for damaged mitochondria leading to the removal of the dysfunctional organelles from the cells by mitophagy [170]. Proteins linked to ubiquitin are digested by the ubiquitin-proteasome system (UPS), allowing clearance of damaged proteins [171]. Mutations in PINK1 or parkin may lead to parkinsonism due to a failure to maintain mitophagy, among other consequences, such as redox impairment and bioenergetics collapse [172]. Lin and Tsai [161] found that CA stimulated mitophagy by activation of the PINK1/parkin axis, preventing the mitochondria-related apoptosis in the 6-OHDA-treated SH-SY5Y cells (Fig. 9). Silencing of PINK1 abrogated the upregulation of parkin and of voltage-dependent anion channel 1 (VDAC1, which is present in the outer membrane of mitochondria and serve as a target of parkin after mitochondrial depolarization) observed in the cells pretreated with CA and challenged with 6-OHDA. Thus, CA promoted neuroprotection by depending on the stimulation of the PINK1/parkin-dependent mitophagy.

Lin *et al.* [162] reported that CA stimulated PINK1 leading to Mic 60 phosphorylation at a threonine residue in SH-SY5Y cells administrated with 6-OHDA. Mic60 is part of the mitochondrial contact site and cristae junction organizing system (MICOS), which is responsible for the

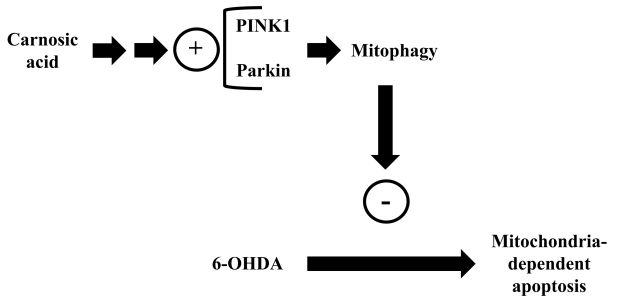


Fig. 9. Carnosic acid stimulates the PINK1/ E3 ubiquitin ligase (parkin) axis causing cytoprotection by a mechanism dependent on mitophagy. Please, read the text for detailed information.

maintenance of mitochondrial architecture regarding cristae plasticity [173]. Moreover, Mic60 also binds to PINK1 and stabilizes this protein on the surface of damaged mitochondria, favoring the binding to parkin and the triggering of mitophagy [174]. Blockade of this interaction leads to mitochondrial impairment and cell death [174]. Furthermore, Mic60 is a target for protein kinase A (PKA), that phosphorylates Mic60 at a serine residue, affecting the interaction of this protein with PINK1 and promoting several negative consequences, including the suppression of the translocation of parkin to the injured mitochondria and the triggering of cytochrome c release to the cytosol [175]. The effects seen by the authors in the cells administrated with CA indicate that this diterpene downregulated PKA, stimulating the Mic60/PINK1-mediated mitophagy and rescuing neurons from the mitochondria-dependent cell death triggered by 6-OHDA (Fig. 10).

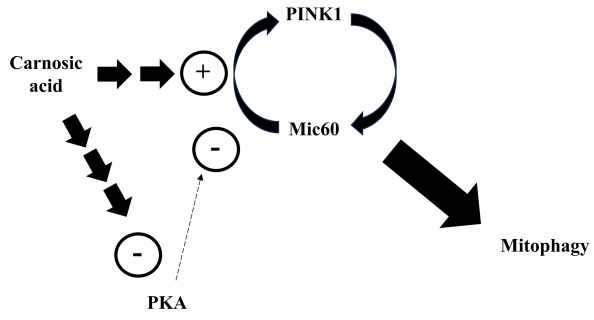


Fig. 10. Carnosic acid stimulates the PINK1 and Mic60 interaction by a mechanism associated with the downregulation of PKA, causing mitophagy and cytoprotection in cultured cells. Please, read the text for detailed information.

Parkin participates in the control of autophagy also by interacting with Beclin1, which is central for the formation of the autophagosome [176]. For example, silencing of Beclin1 blocked the degradation and consequent removal of α-synuclein in the dopaminergic PC12 cell line [177]. In that context, Lin and Tsai [163] have reported that the silencing of parkin abrogated the CA-dependent upregulation in Beclin1 in the 6-OHDA-treated SH-SY5Y cells. Similarly, administration of bafilomycin A1 (an inhibitor of the fusion between the autophagosome and the lysosome) or wortmannin (a compound that blocks the early stage of the formation of the autophagosome) suppressed the anti-apoptotic actions related to the mitochondria promoted by CA in the cells challenged with 6-OHDA. Then, it may be suggested that CA depends on the stimulation of autophagy to prevent cell death triggered by 6-OHDA.

Interestingly, Sun *et al.* [178] demonstrated that Beclin1 can improve mitochondria-associated membranes (MAMs) in the heart of mice submitted to an experimental model of endotoxemia. MAMs may be characterized as regions in which it is observed a close physical connection involving the outer mitochondrial membrane and other biomembranas intracellularly [179]. MAMs are important to the maintenance of communication between the mitochondria and the endoplasmic reticulum necessary to the transport of Ca^{2+} and lipids, for example [180,181]. Nonetheless, it remains to be examined whether CA would be capable to modulate the MAMs-related physiology in brain cells.

Parkin has been considered as a cytoprotective protein also due to its ability to control the apoptosis-related protein in the transforming growth factor-β (TGF-β) signaling pathway (ARTS)/X-liked inhibitor of apoptosis protein (XIAP) pathway [182]. The protein ARTS is inserted in the outer mitochondrial membrane and, in response to pro-apoptotic stimuli, migrates to the cytosol, binding and blocking XIAP, which, in turn, exerts an anti-apoptotic action by inhibiting caspases-3, -7, and -9 [183]. With regard to CA, it was examined whether this diterpene would be capable to counteract the pro-apoptotic effect triggered by 6-OHDA in the SH-SY5Y cells by a mechanism dependent on the parkin/ARTS/XIAP axis. Fu *et al.* [164] observed that a pretreatment with CA attenuated the 6-OHDA-induced ARTS upregulation and XIAP downregulation in the cells treated with 6-OHDA. Moreover, CA prevented the activation of the caspases-9 and -7 in that work. Silencing of parkin blocked the effects promoted by CA on levels and activation of ARTS, XIAP, and caspases-9 and -7 in the cells challenged with 6-OHDA, leading to cell death (Fig. 11). Thus, in addition to the role of parkin in modulating mitophagy, this protein is associated with the mitochondria-dependent ARTS/XIAP axis involved in the control of apoptosis in CA-treated cells. Together, these data indicate a complex mechanism by which CA promotes neuroprotection by both mitochondria-dependent and independent manners.

Mitochondrial dynamics, i.e., fusion (which is the generation of one mitochondrion by the fusion of two mitochondria) and fission (when one mitochondrion divides

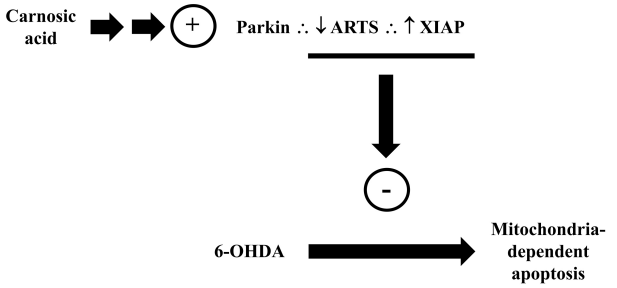


Fig. 11. Carnosic acid modulates the parkin/ARTS/ XIAP signaling pathway blocking the mitochondria-dependent apoptosis induced by the toxicant 6-OHDA. Please, read the text for detailed information.

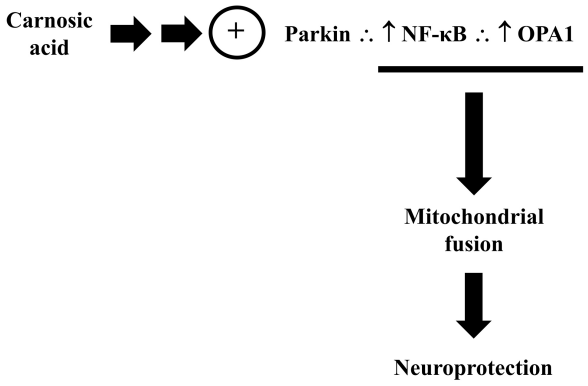


Fig. 12. Carnosic acid modulates the parkin/NF-κB/OPA1 signaling pathway causing mitochondrial fusion and neuroprotection in cultured cells. Please, read the text for detailed information.

generating two mitochondria), alterations have been seen in several disorders, including neurodegeneration and cardiovascular diseases [184,185]. The proteins Drp1 and fission 1 (Fis1) mediates fission, whereas the proteins mitofusin 1 and 2 (MFN1 and MFN2, respectively) and OPA1 modulate mitochondrial fusion [186]. OPA1, which is located in the IMM, has been reported as a target of CA through the stimulation of the parkin/IκB kinase (IKK)/NF-κB signaling pathway [165]. The authors have shown that CA depended on both parkin and OPA1 to block the mitochondria-related pro-apoptotic effects elicited by 6-OHDA in the SH-SY5Y cells. CA upregulated the expression of OPA1 by a mechanism associated with the stimulation of NF-κB, a major transcription factor involved with the maintenance of mitochondrial dynamics [187] (Fig. 12). It is worthy of mention that OPA1 is not associated only with the regulation of protein dynamics, since this protein has been viewed as a neuroprotectant agent in several experimental models [188,189]. Actually, downregulation of OPA1 leads to loss of mitochondrial architecture and, consequently, mitochondrial dysfunction, cytochrome c release and apoptosis in cultured cells [190]. Therefore, CA is a potential neuroprotectant also due to its ability in controlling mitochondrial dynamics and causing pro-survival effects.

In addition to the ability to modulate mitochondrial dynamics, CA also promoted mitochondrial biogenesis in *in vitro* experimental models. Lin *et al.* [166] observed that a pretreatment with CA attenuated the downregulation induced by 6-OHDA on the proteins NRF1 and TFAM, that modulates mitochondrial biogenesis [43,191]. CA also upregulated PGC-1α, a transcription factor that controls the expression of NRF1 and TFAM [43]. In turn, PGC-1α expression is controlled by parkin-interacting substrate (PARIS), a peptide that, under physiological conditions, is ubiquitinated by parkin and follows to degradation by the proteasome [192]. In that context, the authors found that CA attenuated the 6-OHDA-induced upregulation of PARIS by a mechanism associated with parkin. Thus, CA stimulated the parkin/PARIS/PGC-1α pathway leading to mitochondrial biogenesis and mitochondria-related anti-apoptotic actions in the SH-SY5Y cells challenged with 6-OHDA (Fig. 13). Indeed, mitochondrial biogenesis has been viewed as a potential pharmacological target to either prevent or manage neurodegenerative disorders [193].

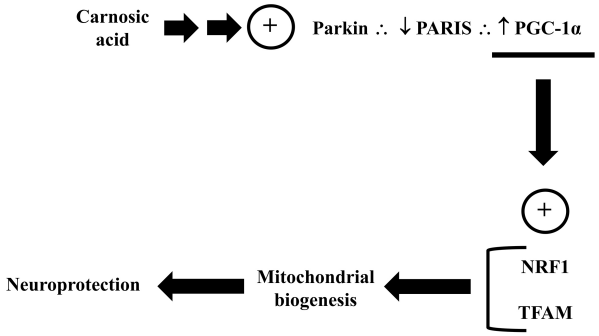


Fig. 13. Carnosic acid regulates the parkin/PARIS/PGC-1α signaling pathway causing the upregulation of the NRF1 and TFAM proteins, which promotes mitochondrial biogenesis in mammalian cells. Please, read the text for detailed information.

3. *In Vivo* Experimental Models

The ability of CA in modulating mitochondria-related parameters in mammalian brain was tested in different experimental models. Prior studies have proved that CA affects the function and dynamics of mitochondria and, therefore, is a potential agent to be utilized as a mitochondria-related protectant in brain disorders.

CA exhibits a potent antioxidant action by modulating both non-enzymatic and enzymatic antioxidant defenses in mammalian cells [89,156]. In a study conducted in aged rats, it was found that supplementation with rosemary ex-

Table 3. Summary of the neuroprotective effects induced by CA in *in vivo* experimental models.

Experimental Model	Dose and Duration	Effects	References
C57BL/6 mice	1 and 3 h CA at 3 mg in 0.3 mL olive oil prior to MCAO	↓ OS, ↑ Nrf2, ↑ GSH	[146]
Wistar rats	20 mg/kg CA treatment for 3 weeks prior to 6-OHDA exposure	↑ GSH, ↓ LPO, ↑ SOD, ↑ GR, ↓ p38, ↑ Bax/Bcl-2 ratio, ↓ cleaved caspase 3/caspase 3, ↓ cleaved PARP/PARP ratio, ↑ TH	[152]
Wistar rats	CA at 20 mg/kg 3 times/3 weeks prior to the administration of 6-OHDA on day 22	↑ PINK1, ↑ Parkin protein, ↓ ubiquitinated protein	[160]
Wistar rats	Rosemary extract 0.2%, 0.02% for 12 weeks	↓ ROS, ↓ LPO, ↓ CAT, ↓ NOS	[194]
SAMP8 mice	60% and 10% CA with 32, 16, 1.6 mg/kg for 16 weeks	↓ 4-HNE, ↓ PCC	[195]
CF-1 mice	1.0 mg/kg CA given intraperitoneally 48 h prior to the exposure of 4-HNE	↑ ADP rate, ↑ succinate rate, ↑ HO-1 mRNA, ↓ 4-HNE	[197]
CF-1 mice	CA at 0.3, 1.0, and 3.0 mg/kg I.P. 15 min post TBI	↓ OS, ↓ LPO, ↓ 3-NT	[198]
Balb/c mice	20 mg/kg/day CA for 3 weeks in bilateral ovariectomy	↑ Nrf2, ↑ HO-1, ↑ thioredoxin-1, ↑ BDNF, ↑ serotonin, ↓ TNF- α, ↓ IL-1β, ↓ iNOS mRNA	[199]
Swiss albino mice	30, 60 mg/kg/day once/day for 14 days before exposure to chlorpyrifos for last 7 days	↓ IL-1β, ↓ TNF-α, ↓ IL-6, ↓ NO, ↓ MDA, ↑ SOD, ↑ GSH, ↑ GPx, ↑ CAT, ↑ AChE	[201]
C57BL/6 mice	CA at 1.0 mg/kg given intraperitoneally 30 min after injury	↑ motor and cognitive function, ↓ GFAP and Iba1	[203]
Wistar rats	CA at 5, 10, 20, and 40 mg/kg injected 30 min prior exposure to acrylamide for 11 days	↑ GSH, ↓ LPO, ↓ Bax/Bcl-2 ratio, ↓ caspase 3 protein levels	[205]
Syrian mice	CA at 10 mg/kg was injected immediately after ischemia-reperfusion and then CA at 3 mg/kg for 10 days	↓ Caspase-3, ↓ Bax, ↑ Bcl-2	[206]
Sprague Dawley rats	3 mg/kg CA given 24 h prior to SAH	↓ ROS, ↓ neural cell death, ↓ p66shc, ↓ Bax, ↓ cleaved caspase-3, ↑ SIRT1, ↑ Mn-SOD, ↑ Bcl-2	[207]

MCAO, middle cerebral artery occlusion; LPO, lipid peroxidation; GR, glutathione reductase; TH, tyrosine hydroxylase; ROS, reactive oxygen species; NOS, nitric oxide synthase; MDA, malondialdehyde; AChE, acetylcholinesterase; TBI, traumatic brain injury; SAH, subarachnoid haemorrhage; OS, oxidative stress; PCC, protein carbonylation; 3-NT, 3-nitrotyrosine; GFAP, glial fibrillary acidic protein; Iba1, ionized calcium-binding adapter molecule 1; CAT, catalase; 4-HNE, 4-hydroxynonenal; BDNF, brain-derived neurotrophic factor; iNOS, inducible nitric oxide synthase; GPx, glutathione peroxidase; SIRT1, NAD+-dependent deacetylase sirtuin 1; i.p., intraperitoneal.

tract containing different concentrations of CA improved antioxidant defenses in the rat brain. Rats exposed to rosemary extract showed decreased levels of ROS, lipid peroxidation (LPO), catalase (CAT), and nitric oxide synthase (NOS), indicating a better redox balance in comparison with the control [194]. In another study, the authors reported that an extract of rosemary presenting 60% CA decreased the levels of 4-hydroxynonenal (4-HNE, a LPO indicator) in the cerebral cortex of the senescence accelerated mice (SAMP8) [195]. The authors also demonstrated that rosemary extract combined with 10% CA significantly attenuated the levels of protein carbonylation in the mice hippocampus.

CA presents a catechol loop that can be oxidized to form an electrophilic quinone [146]. This quinone can interact with particular cysteine bonds on target proteins through thiol *S*-alkylation. The primary response against certain reactive agents, such as ROS and electrophiles, is the Kelch-like ECH-associated protein 1 (Keap1)/Nrf2 pathway [196]. Satoh *et al.* [146] provided the first description of the role of the Nrf2 signaling in mediating the neuroprotective action of CA against oxidative damage and excitotoxicity in an experimental model of middle cerebral artery occlusion (MCAO). The authors demonstrated that the crucial cysteine thiol of the Keap1 protein was alkylated by the electrophilic quinone-type of CA, which triggered the activation of the transcription element Nrf2, and upregulated several cytoprotective enzymes, as commented before.

Numerous studies showed that CA stimulates Nrf2, thus promoting cytoprotective benefits. It was observed that an *in vivo* treatment with CA at 1 mg/kg attenuated the mitochondrial impairment caused by the *in vitro* exposure of rat cerebral cortex to 4-HNE in an *ex vivo* experimental model [197]. The authors also reported that administering CA *in vivo*, reduced the levels of 4-HNE associated with mitochondrial proteins probably by a mechanism related to the stimulation of the Nrf2/ARE/HO-1 pathway. It was also shown that a combination of CA and sulforaphane considerably reduced the induced-4-HNE decline in the respiration rates of mitochondria. CA also attenuated mitochondrial dysfunction in an experimental model of controlled corti-

cal impact (CCI) in mice. Post-administration of 3 mg/kg CA preserved the respiratory action of mitochondria and alleviated oxidative damage by lowering the protein nitration levels, LPO, and cytoskeletal disintegration in the CCI mice [198].

The effects of CA therapy in a recent study included the stimulation of the HO-1, thioredoxin-1, Nrf2, and brain-derived neurotrophic factor (BDNF) expressions, as well as an increase in the brain serotonin levels in ovariectomized mice [199]. In the same study, CA attenuated oxidative stress, as well as caused a decline in the expression of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and inducible NOS (iNOS). Even though Nrf2 and BDNF are linked and it has been documented, leading to antidepressant-like effects in rodents [200], it was not investigated yet regarding the effects caused by CA in the mammalian brain.

Additionally, CA reduced inflammation in mice subjected to the pesticide chlorpyrifos (CPF) [201]. A pretreatment with CA reduced the activity of IL-6, TNF-α, and IL-1β in the serum of the CPF-administered mice. CA also inhibited the CPF-induced decline in the levels of acetylcholinesterase (AChE) in the mice serum. Besides, CA-treated animals showed decreased amounts of $NO^{\bullet}$ and malondialdehyde (MDA) along with increased GSH, glutathione peroxidase (GPx), superoxide dismutase (SOD), and CAT levels in the brain and eye tissues. Similarly, in another study it was observed that protein levels of IL-1β, IL-6, and TNF-α were significantly lowered in the brain of CA-treated mouse submitted to an experimental model of high-fat diet (HFD)-induced inflammation. Treatment with CA attenuated brain injury by upregulating the Bcl-2 protein and downregulating the Bax and matrix metallopeptidase 9 (MMP9) proteins [202]. The neuro-inflammatory markers such as glial fibrillary acidic protein (GFAP) and ionized calcium-binding adapter molecule 1 (Iba1) were found to be decreased with the post-administration of CA in an experimental model of traumatic brain injury (TBI) [203]. In the same study, authors also reported that CA did not affect the changes in mitochondrial respiration after TBI.

The most widely used drug for the development of PD model is 6-OHDA, a neurotoxic organic compound that specifically kills dopaminergic and noradrenergic neurons [204]. It was found that CA increased the GSH levels with decrease in lipid peroxidation levels along with enhanced protein expression of GCLC, GCLM, SOD, and glutathione reductase (GR) in rats exposed to 6-OHDA in PD experimental model. Simultaneously, CA deactivated c-Jun NH2-terminal kinase and p38, upregulated the Bcl-2/Bax ratio, downregulated the cleaved caspase 3/caspase 3 and cleaved PARP/PARP ratios, and upregulated tyrosine hydroxylase (TH) protein in the brain of 6-OHDA lesioned rats [152].

The mitochondria-related anti-apoptotic CA-induced effect has also been demonstrated in an animal model of neurotoxicity induced by acrylamide. Treatment with 40 mg/kg CA for 11 days enhanced the GSH content and lowered the levels of MDA, the Bax/Bcl-2 ratio, and the activation of caspase-3 in the brain of the rats exposed to acrylamide [205]. Another study showed that CA, in combination with Trolox and human chorionic gonadotropin (HCG), reduced the activity of caspase-3 and Bax in the hippocampus of rats administered to an experimental model of ischemia-reperfusion induced by occlusion of the carotid artery [206]. It was also reported that CA upregulated the levels of SIRT1, Mn-SOD, and Bcl-2, while downregulated the expression of p66shc and Bax and the activation of caspase-3 in an experimental model of subarachnoid haemorrhage (SAH) in rats [207].

Overall, it has been demonstrated both CA can modulate mitochondrial physiology by both indirect and direct mechanisms in the brain cells also *in vivo* (Fig. 14; Table 3, Ref. [146,152,160,194,195,197–199,201,203,205–207]). Further studies are necessary to confirm this ability in other contexts involving mitochondrial disturbances.

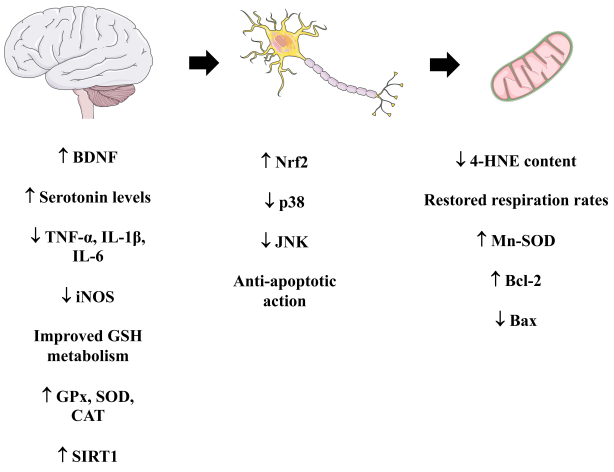


Fig. 14. A summary of the mitochondria-related effects induced by carnosic acid *in vivo*. Please, read the text for detailed information. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license. BDNF, brain-derived neurotrophic factor; iNOS, inducible nitric oxide synthase; GPx, glutathione peroxidase; SOD, superoxide dismutase; CAT, catalase; SIRT1, NAD^{+}-dependent deacetylase sirtuin 1; 4-HNE, 4-hydroxynonenal; JNK, Janus kinase.

4. Conclusion and Future Directions

The constellation of effects induced by CA on the mitochondria clearly demonstrates that this diterpene is a potential candidate to be utilized as a drug in mitochondrial therapy. Even though some of the findings were confirmed in *in vivo* experimental models, further investigations are

necessary to better understand CA bioavailability and to examine whether CA would exert toxic effects in brain cells (as well as in other organs), for example. Moreover, more research needs to be done on this diterpene's potential to alter some signaling pathways connected to mitochondria *in vivo*. Overall, in various brain cells, CA has been shown to be a powerful agent that protects the mitochondria.

Abbreviations

4-HNE, 4-hydroxynonenal; 6-OHDA, 6-hydroxydopamine; AChE, acetylcholinesterase; ACLY, ATP-citrate lyase; AD, Alzheimer's disease; AIF, apoptosis-inducing factor; ALS, amyotrophic lateral sclerosis; AMP, adenosine monophosphate; AMPK, AMP-activated protein kinase; Apaf-1, apoptotic protease activating factor; ARE, antioxidant-responsive element; ARTS, apoptosis-related protein in the TGF-β signaling pathway; ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; BNIP3, BCL2 interacting protein 3; BSO, L-buthionine sulfoximine; BVR, biliverdin reductase; CA, carnosic acid; CAT, catalase; CCI, controlled cortical impact; CO, carbon monoxide; CORM-2, carbon monoxide-releasing molecule-2; CPF, chlorpyrifos; CYP, cytochrome P450; Drp1, dynamin-related protein 1; $FADH_2$, flavin adenine dinucleotide, reduced form; Fis1, fission 1; FUNDC1, FUN14 domain-containing protein 1; GABP, GA-binding protein; γ-GCL, γ-glutamate-cysteine ligase; GCLC, glutamate-cysteine ligase catalytic subunit; GCLM, glutamate-cysteine ligase modifier subunit; GPx, glutathione peroxidase; GR, glutathione reductase; GSH, glutathione, reduced form; GSTP, Pi form of glutathione-*S*-transferase; GTPase, GTP-hydrolyzing proteins; H_2O_2, hydrogen peroxide; HCG, human chorionic gonadotropin; HD, Huntington's disease; HO-1, heme oxygenase-1; IKK, IκB kinase; IMM, inner mitochondrial membrane; iNOS, inducible nitric oxide synthase; IL-1α, interleukin-1α; IL-6, interleukin-6; Keap1, Kelch-like ECH-associated protein 1; MAMs, mitochondria-associated membranes; MAO, monoamine oxidase; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; MICOS, mitochondrial contact site and cristae junction organizing system; MFN1, mitofusin 1; MFN2, mitofusin 2; MMP, mitochondrial membrane potential; MMP9, matrix metallopeptidase 9; Mn-SOD, manganese-dependent superoxide dismutase; mPTP, mitochondrial permeability transition pore; mtDNA, mitochondrial DNA; NADH, nicotinamide adenine dinucleotide; NF-κB, nuclear factor-κB; $NO^{\bullet}$, nitric oxide; NOS, nitric oxide synthase; NQO1, NAD(P)H quinone oxidoreductase-1; NRF1, nuclear respiratory factor 1; Nrf2, nuclear factor erythroid 2-related factor 2; $O_2^{\bullet -}$, radical anion superoxide; $OH^{\bullet}$, hydroxyl radical; OMM, outer mitochondrial membrane; OPA1, optic atrophy 1; OXPHOS, oxidative phosphorylation; PARIS, parkin-interacting substrate; Parkin, E3 ubiquitin ligase; PARP, poly (ADP-ribose) polymerase; PD, Parkinson's disease; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator-1 α; PGE_2, prostaglandin E_2; PI3K, phosphatidylinositol 3-kinase; PINK1, PTEN-induced putative protein kinase 1; PKA, protein kinase A; PPARγ, peroxisome proliferator-activated receptor γ; ROS, reactive oxygen species; SAH, subarachnoid haemorrhage; SAMP8, senescence accelerated mice; SIRT1, NAD^+-dependent deacetylase sirtuin 1; SOD, superoxide dismutase; SQSTM1, sequestosome 1; TBI, traumatic brain injury; TCA, tricarboxylic acid; TFAM, mitochondrial transcription factor A; TH, tyrosine hydroxylase; TNF-α, tumor necrosis factor-α; UPS, ubiquitin-proteasome system; VDAC1, voltage-dependent anion channel 1; XIAP, X-liked inhibitor of apoptosis protein; ZnPP-IX, zinc protoporphyrin-IX.

Author Contributions

Conceptualization: MRdeO, VI, IP, AS, ST, GS; writing—original draft preparation: VI, IP, AS, ST, GS, MRdeO; writing—review and editing: VI, IP, AS, ST, GS, MRdeO. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

MRdeO receives a "Bolsa de Produtividade em Pesquisa (Research Productivity Grant) 2—PQ2" fellow from the CNPq (protocol number 301273/2018-9).

Conflict of Interest

The authors declare no conflict of interest. Marcos Roberto de Oliveira is serving as one of the Guest editors of this journal. We declare that Marcos Roberto de Oliveira had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Gernot Riedel.

References

[1] Frey TG, Mannella CA. The internal structure of mitochondria. Trends in Biochemical Sciences. 2000; 25: 319–324.

[2] Reid PC, Urano Y, Kodama T, Hamakubo T. Alzheimer's disease: cholesterol, membrane rafts, isoprenoids and statins. Journal of Cellular and Molecular Medicine. 2007; 11: 383–392.

[3] Rizzuto R, De Stefani D, Raffaello A, Mammucari C. Mitochondria as sensors and regulators of calcium signalling. Nature Reviews. Molecular Cell Biology. 2012; 13: 566–578.

[4] Murali M, Carvalho MS, Shivanandappa T. Oxidative stress-mediated cytotoxicity of Endosulfan is causally linked to the inhibition of NADH dehydrogenase and Na^+, K^+-ATPase in Ehrlich ascites tumor cells. Molecular and Cellular Biochemistry. 2020; 468: 59–68.
[5] Pilchova I, Klacanova K, Tatarkova Z, Kaplan P, Racay P. The Involvement of Mg^{2+} in Regulation of Cellular and Mitochondrial Functions. Oxidative Medicine and Cellular Longevity. 2017; 2017: 6797460.
[6] Angajala A, Lim S, Phillips JB, Kim JH, Yates C, You Z, *et al*. Diverse Roles of Mitochondria in Immune Responses: Novel Insights Into Immuno-Metabolism. Frontiers in Immunology. 2018; 9: 1605.
[7] Breda CNDS, Davanzo GG, Basso PJ, Saraiva Câmara NO, Moraes-Vieira PMM. Mitochondria as central hub of the immune system. Redox Biology. 2019; 26: 101255.
[8] Golpich M, Amini E, Mohamed Z, Azman Ali R, Mohamed Ibrahim N, Ahmadiani A. Mitochondrial Dysfunction and Biogenesis in Neurodegenerative diseases: Pathogenesis and Treatment. CNS Neuroscience & Therapeutics. 2017; 23: 5–22.
[9] Wang Y, Xu E, Musich PR, Lin F. Mitochondrial dysfunction in neurodegenerative diseases and the potential countermeasure. CNS Neuroscience & Therapeutics. 2019; 25: 816–824.
[10] Varughese JT, Buchanan SK, Pitt AS. The Role of Voltage-Dependent Anion Channel in Mitochondrial Dysfunction and Human Disease. Cells. 2021; 10: 1737.
[11] Gyimesi G, Hediger MA. Sequence Features of Mitochondrial Transporter Protein Families. Biomolecules. 2020; 10: 1611.
[12] Kühlbrandt W. Structure and function of mitochondrial membrane protein complexes. BMC Biology. 2015; 13: 89.
[13] Infantino V, Todisco S, Convertini P. 1 - Mitochondrial physiology: An overview. In de Oliveira MR (ed.) Mitochondrial Intoxication (pp. 1–27). Academic Press: San Diego, CA, USA. 2023.
[14] Taanman JW. The mitochondrial genome: structure, transcription, translation and replication. Biochimica et Biophysica Acta. 1999; 1410: 103–123.
[15] D'Souza AR, Minczuk M. Mitochondrial transcription and translation: overview. Essays in Biochemistry. 2018; 62: 309–320.
[16] Papa S, Martino PL, Capitanio G, Gaballo A, De Rasmo D, Signorile A, *et al*. The oxidative phosphorylation system in mammalian mitochondria. Advances in Experimental Medicine and Biology. 2012; 942: 3–37.
[17] Owen OE, Kalhan SC, Hanson RW. The key role of anaplerosis and cataplerosis for citric acid cycle function. The Journal of Biological Chemistry. 2002; 277: 30409–30412.
[18] Martínez-Reyes I, Chandel NS. Mitochondrial TCA cycle metabolites control physiology and disease. Nature Communications. 2020; 11: 102.
[19] Williams NC, O'Neill LAJ. A Role for the Krebs Cycle Intermediate Citrate in Metabolic Reprogramming in Innate Immunity and Inflammation. Frontiers in Immunology. 2018; 9: 141.
[20] Infantino V, Iacobazzi V, Menga A, Avantaggiati ML, Palmieri F. A key role of the mitochondrial citrate carrier (SLC25A1) in TNFα- and IFNγ-triggered inflammation. Biochimica et Biophysica Acta. 2014; 1839: 1217–1225.
[21] Infantino V, Convertini P, Cucci L, Panaro MA, Di Noia MA, Calvello R, *et al*. The mitochondrial citrate carrier: a new player in inflammation. The Biochemical Journal. 2011; 438: 433–436.
[22] Choi I, Son H, Baek JH. Tricarboxylic Acid (TCA) Cycle Intermediates: Regulators of Immune Responses. Life. 2021; 11: 69.
[23] Salminen A, Kauppinen A, Hiltunen M, Kaarniranta K. Krebs cycle intermediates regulate DNA and histone methylation: epigenetic impact on the aging process. Ageing Research Reviews. 2014; 16: 45–65.
[24] Murphy MP. How mitochondria produce reactive oxygen species. The Biochemical Journal. 2009; 417: 1–13.
[25] Mailloux RJ. An Update on Mitochondrial Reactive Oxygen Species Production. Antioxidants. 2020; 9: 472.
[26] Andreyev AY, Kushnareva YE, Starkov AA. Mitochondrial metabolism of reactive oxygen species. Biochemistry. Biokhimiia. 2005; 70: 200–214.
[27] Yasui H, Hayashi S, Sakurai H. Possible involvement of singlet oxygen species as multiple oxidants in p450 catalytic reactions. Drug Metabolism and Pharmacokinetics. 2005; 20: 1–13.
[28] Kaludercic N, Mialet-Perez J, Paolocci N, Parini A, Di Lisa F. Monoamine oxidases as sources of oxidants in the heart. Journal of Molecular and Cellular Cardiology. 2014; 73: 34–42.
[29] Milkovic L, Cipak Gasparovic A, Cindric M, Mouthuy PA, Zarkovic N. Short Overview of ROS as Cell Function Regulators and Their Implications in Therapy Concepts. Cells. 2019; 8: 793.
[30] Shields HJ, Traa A, Van Raamsdonk JM. Beneficial and Detrimental Effects of Reactive Oxygen Species on Lifespan: A Comprehensive Review of Comparative and Experimental Studies. Frontiers in Cell and Developmental Biology. 2021; 9: 628157.
[31] Marí M, Morales A, Colell A, García-Ruiz C, Fernández-Checa JC. Mitochondrial glutathione, a key survival antioxidant. Antioxidants & Redox Signaling. 2009; 11: 2685–2700.
[32] Ham AJ, Liebler DC. Vitamin E oxidation in rat liver mitochondria. Biochemistry. 1995; 34: 5754–5761.
[33] Rabilloud T, Heller M, Rigobello MP, Bindoli A, Aebersold R, Lunardi J. The mitochondrial antioxidant defence system and its response to oxidative stress. Proteomics. 2001; 1: 1105–1110.
[34] Elmore S. Apoptosis: a review of programmed cell death. Toxicologic Pathology. 2007; 35: 495–516.
[35] Dorstyn L, Akey CW, Kumar S. New insights into apoptosome structure and function. Cell Death and Differentiation. 2018; 25: 1194–1208.
[36] Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. Nature Reviews. Cancer. 2002; 2: 647–656.
[37] Tian C, Liu Y, Li Z, Zhu P, Zhao M. Mitochondria Related Cell Death Modalities and Disease. Frontiers in Cell and Developmental Biology. 2022; 10: 832356.
[38] Li Q, Shi N, Cai C, Zhang M, He J, Tan Y, *et al*. The Role of Mitochondria in Pyroptosis. Frontiers in Cell and Developmental Biology. 2021; 8: 630771.
[39] Gao M, Yi J, Zhu J, Minikes AM, Monian P, Thompson CB, *et al*. Role of Mitochondria in Ferroptosis. Molecular Cell. 2019; 73: 354–363.e3.
[40] Palikaras K, Tavernarakis N. Mitochondrial homeostasis: the interplay between mitophagy and mitochondrial biogenesis. Experimental Gerontology. 2014; 56: 182–188.
[41] Ploumi C, Daskalaki I, Tavernarakis N. Mitochondrial biogenesis and clearance: a balancing act. The FEBS Journal. 2017; 284: 183–195.
[42] Sanchis-Gomar F, García-Giménez JL, Gómez-Cabrera MC, Pallardó FV. Mitochondrial biogenesis in health and disease. Molecular and therapeutic approaches. Current Pharmaceutical Design. 2014; 20: 5619–5633.
[43] Popov LD. Mitochondrial biogenesis: An update. Journal of Cellular and Molecular Medicine. 2020; 24: 4892–4899.
[44] Yang ZF, Drumea K, Mott S, Wang J, Rosmarin AG. GABP transcription factor (nuclear respiratory factor 2) is required for mitochondrial biogenesis. Molecular and Cellular Biology. 2014; 34: 3194–3201.
[45] Rosmarin AG, Resendes KK, Yang Z, McMillan JN, Fleming SL. GA-binding protein transcription factor: a review of GABP as an integrator of intracellular signaling and protein-protein in-

teractions. Blood Cells, Molecules & Diseases. 2004; 32: 143–154.
[46] Liang H, Ward WF. PGC-1alpha: a key regulator of energy metabolism. Advances in Physiology Education. 2006; 30: 145–151.
[47] Larsson NG, Wang J, Wilhelmsson H, Oldfors A, Rustin P, Lewandoski M, *et al*. Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. Nature Genetics. 1998; 18: 231–236.
[48] Asin-Cayuela J, Gustafsson CM. Mitochondrial transcription and its regulation in mammalian cells. Trends in Biochemical Sciences. 2007; 32: 111–117.
[49] Canugovi C, Maynard S, Bayne ACV, Sykora P, Tian J, de Souza-Pinto NC, *et al*. The mitochondrial transcription factor A functions in mitochondrial base excision repair. DNA Repair. 2010; 9: 1080–1089.
[50] Cantó C, Gerhart-Hines Z, Feige JN, Lagouge M, Noriega L, Milne JC, *et al*. AMPK regulates energy expenditure by modulating NAD+ metabolism and SIRT1 activity. Nature. 2009; 458: 1056–1060.
[51] Dorn GW, 2nd, Vega RB, Kelly DP. Mitochondrial biogenesis and dynamics in the developing and diseased heart. Genes & Development. 2015; 29: 1981–1991.
[52] Sheng B, Wang X, Su B, Lee HG, Casadesus G, Perry G, *et al*. Impaired mitochondrial biogenesis contributes to mitochondrial dysfunction in Alzheimer's disease. Journal of Neurochemistry. 2012; 120: 419–429.
[53] Kim I, Rodriguez-Enriquez S, Lemasters JJ. Selective degradation of mitochondria by mitophagy. Archives of Biochemistry and Biophysics. 2007; 462: 245–253.
[54] Iorio R, Celenza G, Petricca S. Mitophagy: Molecular Mechanisms, New Concepts on Parkin Activation and the Emerging Role of AMPK/ULK1 Axis. Cells. 2021; 11: 30.
[55] Durcan TM, Fon EA. The three 'P's of mitophagy: PARKIN, PINK1, and post-translational modifications. Genes & Development. 2015; 29: 989–999.
[56] Poon A, Saini H, Sethi S, O'Sullivan GA, Plun-Favreau H, Wray S, *et al*. The role of SQSTM1 (p62) in mitochondrial function and clearance in human cortical neurons. Stem Cell Reports. 2021; 16: 1276–1289.
[57] Lampert MA, Orogo AM, Najor RH, Hammerling BC, Leon LJ, Wang BJ, *et al*. BNIP3L/NIX and FUNDC1-mediated mitophagy is required for mitochondrial network remodeling during cardiac progenitor cell differentiation. Autophagy. 2019; 15: 1182–1198.
[58] Wang J, Zhu P, Li R, Ren J, Zhou H. Fundc1-dependent mitophagy is obligatory to ischemic preconditioning-conferred renoprotection in ischemic AKI via suppression of Drp1-mediated mitochondrial fission. Redox Biology. 2020; 30: 101415.
[59] Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death and Differentiation. 2013; 20: 31–42.
[60] De Gaetano A, Gibellini L, Zanini G, Nasi M, Cossarizza A, Pinti M. Mitophagy and Oxidative Stress: The Role of Aging. Antioxidants. 2021; 10: 794.
[61] Suen DF, Norris KL, Youle RJ. Mitochondrial dynamics and apoptosis. Genes & Development. 2008; 22: 1577–1590.
[62] Scott I, Youle RJ. Mitochondrial fission and fusion. Essays in Biochemistry. 2010; 47: 85–98.
[63] Ni HM, Williams JA, Ding WX. Mitochondrial dynamics and mitochondrial quality control. Redox Biology. 2015; 4: 6–13.
[64] Song M, Mihara K, Chen Y, Scorrano L, Dorn GW, 2nd. Mitochondrial fission and fusion factors reciprocally orchestrate mitophagic culling in mouse hearts and cultured fibroblasts. Cell Metabolism. 2015; 21: 273–286.
[65] Ishihara N, Eura Y, Mihara K. Mitofusin 1 and 2 play distinct roles in mitochondrial fusion reactions via GTPase activity. Journal of Cell Science. 2004; 117: 6535–6546.
[66] Cipolat S, Martins de Brito O, Dal Zilio B, Scorrano L. OPA1 requires mitofusin 1 to promote mitochondrial fusion. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101: 15927–15932.
[67] Labrousse AM, Zappaterra MD, Rube DA, van der Bliek AM. C. elegans dynamin-related protein DRP-1 controls severing of the mitochondrial outer membrane. Molecular Cell. 1999; 4: 815–826.
[68] Kumar A, Sidhu J, Goyal A, Tsao JW. Alzheimer Disease. Disclosure: Jaskirat Sidhu declares no relevant financial relationships with ineligible companies. Disclosure: Amandeep Goyal declares no relevant financial relationships with ineligible companies. Disclosure: Jack Tsao declares no relevant financial relationships with ineligible companies. In StatPearls: Treasure Island, FL, USA. 2023.
[69] Hyman BT, Phelps CH, Beach TG, Bigio EH, Cairns NJ, Carrillo MC, *et al*. National Institute on Aging-Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. Alzheimer's & Dementia. 2012; 8: 1–13.
[70] Tönnies E, Trushina E. Oxidative Stress, Synaptic Dysfunction, and Alzheimer's Disease. Journal of Alzheimer's Disease. 2017; 57: 1105–1121.
[71] Kerr JS, Adriaanse BA, Greig NH, Mattson MP, Cader MZ, Bohr VA, *et al*. Mitophagy and Alzheimer's Disease: Cellular and Molecular Mechanisms. Trends in Neurosciences. 2017; 40: 151–166.
[72] Quntanilla RA, Tapia-Monsalves C. The Role of Mitochondrial Impairment in Alzheimer´s Disease Neurodegeneration: The Tau Connection. Current Neuropharmacology. 2020; 18: 1076–1091.
[73] Szeto HH. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. British Journal of Pharmacology. 2014; 171: 2029–2050.
[74] Rice AC, Keeney PM, Algarzae NK, Ladd AC, Thomas RR, Bennett JP, Jr. Mitochondrial DNA copy numbers in pyramidal neurons are decreased and mitochondrial biogenesis transcriptome signaling is disrupted in Alzheimer's disease hippocampi. Journal of Alzheimer's Disease. 2014; 40: 319–330.
[75] Manczak M, Calkins MJ, Reddy PH. Impaired mitochondrial dynamics and abnormal interaction of amyloid beta with mitochondrial protein Drp1 in neurons from patients with Alzheimer's disease: implications for neuronal damage. Human Molecular Genetics. 2011; 20: 2495–2509.
[76] Parker WD, Jr, Filley CM, Parks JK. Cytochrome oxidase deficiency in Alzheimer's disease. Neurology. 1990; 40: 1302–1303.
[77] Wilkins HM, Koppel SJ, Bothwell R, Mahnken J, Burns JM, Swerdlow RH. Platelet cytochrome oxidase and citrate synthase activities in APOE ε4 carrier and non-carrier Alzheimer's disease patients. Redox Biology. 2017; 12: 828–832.
[78] Toulorge D, Schapira AHV, Hajj R. Molecular changes in the postmortem parkinsonian brain. Journal of Neurochemistry. 2016; 139: 27–58.
[79] Kamp F, Exner N, Lutz AK, Wender N, Hegermann J, Brunner B, *et al*. Inhibition of mitochondrial fusion by α-synuclein is rescued by PINK1, Parkin and DJ-1. The EMBO Journal. 2010; 29: 3571–3589.
[80] Park JS, Davis RL, Sue CM. Mitochondrial Dysfunction in Parkinson's Disease: New Mechanistic Insights and Therapeutic Perspectives. Current Neurology and Neuroscience Reports. 2018; 18: 21.
[81] Grünewald A, Kumar KR, Sue CM. New insights into the complex role of mitochondria in Parkinson's disease. Progress in

Neurobiology. 2019; 177: 73–93.
[82] Arenas J, Campos Y, Ribacoba R, Martín MA, Rubio JC, Ablanedo P, *et al*. Complex I defect in muscle from patients with Huntington's disease. Annals of Neurology. 1998; 43: 397–400.
[83] Brennan WA, Jr, Bird ED, Aprille JR. Regional mitochondrial respiratory activity in Huntington's disease brain. Journal of Neurochemistry. 1985; 44: 1948–1950.
[84] Naseri NN, Bonica J, Xu H, Park LC, Arjomand J, Chen Z, *et al*. Novel Metabolic Abnormalities in the Tricarboxylic Acid Cycle in Peripheral Cells From Huntington's Disease Patients. PLoS ONE. 2016; 11: e0160384.
[85] Kim J, Moody JP, Edgerly CK, Bordiuk OL, Cormier K, Smith K, *et al*. Mitochondrial loss, dysfunction and altered dynamics in Huntington's disease. Human Molecular Genetics. 2010; 19: 3919–3935.
[86] Johri A, Chandra A, Flint Beal M. PGC-1α, mitochondrial dysfunction, and Huntington's disease. Free Radical Biology & Medicine. 2013; 62: 37–46.
[87] Martinez-Vicente M, Talloczy Z, Wong E, Tang G, Koga H, Kaushik S, *et al*. Cargo recognition failure is responsible for inefficient autophagy in Huntington's disease. Nature Neuroscience. 2010; 13: 567–576.
[88] Zhang X, Wang L, Li B, Shi J, Xu J, Yuan M. Targeting Mitochondrial Dysfunction in Neurodegenerative Diseases: Expanding the Therapeutic Approaches by Plant-Derived Natural Products. Pharmaceuticals. 2023; 16: 277.
[89] Birtić S, Dussort P, Pierre FX, Bily AC, Roller M. Carnosic acid. Phytochemistry. 2015; 115: 9–19.
[90] Mirza FJ, Zahid S, Holsinger RMD. Neuroprotective Effects of Carnosic Acid: Insight into Its Mechanisms of Action. Molecules. 2023; 28: 2306.
[91] Bahri S, Jameleddine S, Shlyonsky V. Relevance of carnosic acid to the treatment of several health disorders: Molecular targets and mechanisms. Biomedicine & Pharmacotherapy. 2016; 84: 569–582.
[92] Loussouarn M, Krieger-Liszkay A, Svilar L, Bily A, Birtić S, Havaux M. Carnosic Acid and Carnosol, Two Major Antioxidants of Rosemary, Act through Different Mechanisms. Plant Physiology. 2017; 175: 1381–1394.
[93] Pavić V, Jakovljević M, Molnar M, Jokić S. Extraction of Carnosic Acid and Carnosol from Sage (Salvia officinalis L.) Leaves by Supercritical Fluid Extraction and Their Antioxidant and Antibacterial Activity. Plants. 2019; 8: 16.
[94] Munné-Bosch S, Alegre L. Subcellular compartmentation of the diterpene carnosic acid and its derivatives in the leaves of rosemary. Plant Physiology. 2001; 125: 1094–1102.
[95] del Baño MJ, Lorente J, Castillo J, Benavente-García O, del Río JA, Ortuño A, *et al*. Phenolic diterpenes, flavones, and rosmarinic acid distribution during the development of leaves, flowers, stems, and roots of Rosmarinus officinalis. Antioxidant activity. Journal of Agricultural and Food Chemistry. 2003; 51: 4247–4253.
[96] Hidalgo PJ, Ubera JL, Tena MT, Valcárcel M. Determination of the Carnosic Acid Content in Wild and Cultivated Rosmarinus officinalis. Journal of Agricultural and Food Chemistry. 1998; 46: 2624–2627.
[97] Brückner K, Božić D, Manzano D, Papaefthimiou D, Pateraki I, Scheler U, *et al*. Characterization of two genes for the biosynthesis of abietane-type diterpenes in rosemary (Rosmarinus officinalis) glandular trichomes. Phytochemistry. 2014; 101: 52–64.
[98] Božić D, Papaefthimiou D, Brückner K, de Vos RCH, Tsoleridis CA, Katsarou D, *et al*. Towards Elucidating Carnosic Acid Biosynthesis in Lamiaceae: Functional Characterization of the Three First Steps of the Pathway in Salvia fruticosa and Rosmarinus officinalis. PLoS ONE. 2015; 10: e0124106.
[99] Doolaege EHA, Raes K, De Vos F, Verhé R, De Smet S. Absorption, distribution and elimination of carnosic acid, a natural antioxidant from Rosmarinus officinalis, in rats. Plant Foods for Human Nutrition. 2011; 66: 196–202.
[100] Romo Vaquero M, García Villalba R, Larrosa M, Yáñez-Gascón MJ, Fromentin E, Flanagan J, *et al*. Bioavailability of the major bioactive diterpenoids in a rosemary extract: metabolic profile in the intestine, liver, plasma, and brain of Zucker rats. Molecular Nutrition & Food Research. 2013; 57: 1834–1846.
[101] Wang QL, Li H, Li XX, Cui CY, Wang R, Yu NX, *et al*. Acute and 30-day oral toxicity studies of administered carnosic acid. Food and Chemical Toxicology. 2012; 50: 4348–4355.
[102] Andrade JM, Faustino C, Garcia C, Ladeiras D, Reis CP, Rijo P. *Rosmarinus officinalis* L.: an update review of its phytochemistry and biological activity. Future Science OA. 2018; 4: FSO283.
[103] Hopia AI, Huang S-W, Schwarz K, German JB, Frankel EN. Effect of Different Lipid Systems on Antioxidant Activity of Rosemary Constituents Carnosol and Carnosic Acid with and without α-Tocopherol. Journal of Agricultural and Food Chemistry. 1996; 44: 2030–2036.
[104] Huang S-W, Frankel EN, Schwarz K, Aeschbach R, German JB. Antioxidant Activity of Carnosic Acid and Methyl Carnosate in Bulk Oils and Oil-in-Water Emulsions. Journal of Agricultural and Food Chemistry. 1996; 44: 2951–2956.
[105] Cuvelier M-E, Richard H, Berset C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. Journal of the American Oil Chemists' Society. 1996; 73: 645–652.
[106] Pearson DA, Frankel EN, Aeschbach R, German JB. Inhibition of Endothelial Cell-Mediated Oxidation of Low-Density Lipoprotein by Rosemary and Plant Phenolics. Journal of Agricultural and Food Chemistry. 1997; 45: 578–582.
[107] Wijeratne SSK, Cuppett SL. Potential of rosemary (Rosemarinus officinalis L.) diterpenes in preventing lipid hydroperoxide-mediated oxidative stress in Caco-2 cells. Journal of Agricultural and Food Chemistry. 2007; 55: 1193–1199.
[108] Aruoma OI, Halliwell B, Aeschbach R, Löligers J. Antioxidant and pro-oxidant properties of active rosemary constituents: carnosol and carnosic acid. Xenobiotica. 1992; 22: 257–268.
[109] Erkan N, Ayranci G, Ayranci E. Antioxidant activities of rosemary (Rosmarinus Officinalis L.) extract, blackseed (Nigella sativa L.) essential oil, carnosic acid, rosmarinic acid and sesamol. Food Chemistry. 2008; 110: 76–82.
[110] Zhang Y, Yang L, Zu Y, Chen X, Wang F, Liu F. Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. Food Chemistry. 2010; 118: 656–662.
[111] Naveena BM, Vaithiyanathan S, Muthukumar M, Sen AR, Kumar YP, Kiran M, *et al*. Relationship between the solubility, dosage and antioxidant capacity of carnosic acid in raw and cooked ground buffalo meat patties and chicken patties. Meat Science. 2013; 95: 195–202.
[112] Jordán MJ, Castillo J, Bañón S, Martínez-Conesa C, Sotomayor JA. Relevance of the carnosic acid/carnosol ratio for the level of rosemary diterpene transfer and for improving lamb meat antioxidant status. Food Chemistry. 2014; 151: 212–218.
[113] Bernardes WA, Lucarini R, Tozatti MG, Souza MGM, Silva MLA, Filho AADS, *et al*. Antimicrobial activity of Rosmarinus officinalis against oral pathogens: relevance of carnosic acid and carnosol. Chemistry & Biodiversity. 2010; 7: 1835–1840.
[114] Del Campo J, Amiot MJ, Nguyen-The C. Antimicrobial effect of rosemary extracts. Journal of Food Protection. 2000; 63: 1359–1368.
[115] Campo JD, Nguyen-The C, Sergent M, Amiot MJ. Determination of the Most Bioactive Phenolic Compounds from Rosemary Against Listeria Monocytogenes: Influence of Concentration,

pH, and NaCl. Journal of Food Science. 2003; 68: 2066–2071.
[116] Horiuchi K, Shiota S, Kuroda T, Hatano T, Yoshida T, Tsuchiya T. Potentiation of antimicrobial activity of aminoglycosides by carnosol from Salvia officinalis. Biological & Pharmaceutical Bulletin. 2007; 30: 287–290.
[117] Moreno S, Scheyer T, Romano CS, Vojnov AA. Antioxidant and antimicrobial activities of rosemary extracts linked to their polyphenol composition. Free Radical Research. 2006; 40: 223–231.
[118] Rožman T, Jeršek B. Antimicrobial activity of rosemary extracts (Rosmarinus officinalis L.) against different species of Listeria. Acta Agriculturae Slovenica. 2009; 93: 51–58.
[119] Klancnik A, Guzej B, Kolar MH, Abramovic H, Mozina SS. In vitro antimicrobial and antioxidant activity of commercial rosemary extract formulations. Journal of Food Protection. 2009; 72: 1744–1752.
[120] Klancnik A, Piskernik S, Jersek B, Mozina SS. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. Journal of Microbiological Methods. 2010; 81: 121–126.
[121] Souza AB, de Souza MGM, Moreira MA, Moreira MR, Furtado NAJC, Martins CHG, *et al*. Antimicrobial evaluation of diterpenes from Copaifera langsdorffii oleoresin against periodontal anaerobic bacteria. Molecules. 2011; 16: 9611–9619.
[122] Ojeda-Sana AM, Repetto V, Moreno S. Carnosic acid is an efflux pumps modulator by dissipation of the membrane potential in Enterococcus faecalis and Staphylococcus aureus. World Journal of Microbiology & Biotechnology. 2013; 29: 137–144.
[123] Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews. 1999; 12: 564–582.
[124] Tsai CW, Lin CY, Wang YJ. Carnosic acid induces the NAD(P)H: quinone oxidoreductase 1 expression in rat clone 9 cells through the p38/nuclear factor erythroid-2 related factor 2 pathway. The Journal of Nutrition. 2011; 141: 2119–2125.
[125] Kontogianni VG, Tomic G, Nikolic I, Nerantzaki AA, Sayyad N, Stosic-Grujicic S, *et al*. Phytochemical profile of Rosmarinus officinalis and Salvia officinalis extracts and correlation to their antioxidant and anti-proliferative activity. Food Chemistry. 2013; 136: 120–129.
[126] López-Jiménez A, García-Caballero M, Medina MÁ, Quesada AR. Anti-angiogenic properties of carnosol and carnosic acid, two major dietary compounds from rosemary. European Journal of Nutrition. 2013; 52: 85–95.
[127] Costa S, Utan A, Speroni E, Cervellati R, Piva G, Prandini A, *et al*. Carnosic acid from rosemary extracts: a potential chemoprotective agent against aflatoxin B1. An in vitro study. Journal of Applied Toxicology. 2007; 27: 152–159.
[128] Lee JJ, Jin YR, Lee JH, Yu JY, Han XH, Oh KW, *et al*. Antiplatelet activity of carnosic acid, a phenolic diterpene from Rosmarinus officinalis. Planta Medica. 2007; 73: 121–127.
[129] Oh J, Yu T, Choi SJ, Yang Y, Baek HS, An SA, *et al*. Syk/Src pathway-targeted inhibition of skin inflammatory responses by carnosic acid. Mediators of Inflammation. 2012; 2012: 781375.
[130] Theoduloz C, Pertino MW, Rodríguez JA, Schmeda-Hirschmann G. Gastroprotective effect and cytotoxicity of carnosic acid derivatives. Planta Medica. 2011; 77: 882–887.
[131] Kocak C, Kocak FE, Akcilar R, Isiklar OO, Kocak H, Bayat Z, *et al*. Molecular and biochemical evidence on the protective effects of embelin and carnosic acid in isoproterenol-induced acute myocardial injury in rats. Life Sciences. 2016; 147: 15–23.
[132] Yu YM, Lin HC, Chang WC. Carnosic acid prevents the migration of human aortic smooth muscle cells by inhibiting the activation and expression of matrix metalloproteinase-9. The British Journal of Nutrition. 2008; 100: 731–738.
[133] Meng P, Yoshida H, Matsumiya T, Imaizumi T, Tanji K, Xing F, *et al*. Carnosic acid suppresses the production of amyloid-β 1-42 by inducing the metalloprotease gene TACE/ADAM17 in SH-SY5Y human neuroblastoma cells. Neuroscience Research. 2013; 75: 94–102.
[134] Meng P, Yoshida H, Tanji K, Matsumiya T, Xing F, Hayakari R, *et al*. Carnosic acid attenuates apoptosis induced by amyloid-β 1-42 or 1-43 in SH-SY5Y human neuroblastoma cells. Neuroscience Research. 2015; 94: 1–9.
[135] Dickmann LJ, VandenBrink BM, Lin YS. In vitro hepatotoxicity and cytochrome P450 induction and inhibition characteristics of carnosic acid, a dietary supplement with antiadipogenic properties. Drug Metabolism and Disposition. 2012; 40: 1263–1267.
[136] Ibarra A, Cases J, Roller M, Chiralt-Boix A, Coussaert A, Ripoll C. Carnosic acid-rich rosemary (Rosmarinus officinalis L.) leaf extract limits weight gain and improves cholesterol levels and glycaemia in mice on a high-fat diet. The British Journal of Nutrition. 2011; 106: 1182–1189.
[137] Aoyagi Y, Takahashi Y, Satake Y, Takeya K, Aiyama R, Matsuzaki T, *et al*. Cytotoxicity of abietane diterpenoids from Perovskia abrotanoides and of their semisynthetic analogues. Bioorganic & Medicinal Chemistry. 2006; 14: 5285–5291.
[138] Sharabani H, Izumchenko E, Wang Q, Kreinin R, Steiner M, Barvish Z, *et al*. Cooperative antitumor effects of vitamin D3 derivatives and rosemary preparations in a mouse model of myeloid leukemia. International Journal of Cancer. 2006; 118: 3012–3021.
[139] Nabekura T, Yamaki T, Hiroi T, Ueno K, Kitagawa S. Inhibition of anticancer drug efflux transporter P-glycoprotein by rosemary phytochemicals. Pharmacological Research. 2010; 61: 259–263.
[140] Ngo SNT, Williams DB, Head RJ. Rosemary and cancer prevention: preclinical perspectives. Critical Reviews in Food Science and Nutrition. 2011; 51: 946–954.
[141] Barni MV, Carlini MJ, Cafferata EG, Puricelli L, Moreno S. Carnosic acid inhibits the proliferation and migration capacity of human colorectal cancer cells. Oncology Reports. 2012; 27: 1041–1048.
[142] Bauer J, Kuehnl S, Rollinger JM, Scherer O, Northoff H, Stuppner H, *et al*. Carnosol and carnosic acids from Salvia officinalis inhibit microsomal prostaglandin E2 synthase-1. The Journal of Pharmacology and Experimental Therapeutics. 2012; 342: 169–176.
[143] Wang T, Takikawa Y, Tabuchi T, Satoh T, Kosaka K, Suzuki K. Carnosic acid (CA) prevents lipid accumulation in hepatocytes through the EGFR/MAPK pathway. Journal of Gastroenterology. 2012; 47: 805–813.
[144] de Oliveira MR. Carnosic Acid as a Promising Agent in Protecting Mitochondria of Brain Cells. Molecular Neurobiology. 2018; 55: 6687–6699.
[145] Wei J, Liang Q, Guo Y, Zhang W, Wu L. A Deep Insight in the Antioxidant Property of Carnosic Acid: From Computational Study to Experimental Analysis. Foods. 2021; 10: 2279.
[146] Satoh T, Kosaka K, Itoh K, Kobayashi A, Yamamoto M, Shimojo Y, *et al*. Carnosic acid, a catechol-type electrophilic compound, protects neurons both in vitro and in vivo through activation of the Keap1/Nrf2 pathway via S-alkylation of targeted cysteines on Keap1. Journal of Neurochemistry. 2008; 104: 1116–1131.
[147] Kosaka K, Mimura J, Itoh K, Satoh T, Shimojo Y, Kitajima C, *et al*. Role of Nrf2 and p62/ZIP in the neurite outgrowth by carnosic acid in PC12h cells. Journal of Biochemistry. 2010; 147: 73–81.
[148] Tamaki Y, Tabuchi T, Takahashi T, Kosaka K, Satoh T. Activated glutathione metabolism participates in protective effects of carnosic acid against oxidative stress in neuronal HT22 cells. Planta Medica. 2010; 76: 683–688.
[149] Chen JH, Ou HP, Lin CY, Lin FJ, Wu CR, Chang SW, *et al*.

Carnosic acid prevents 6-hydroxydopamine-induced cell death in SH-SY5Y cells via mediation of glutathione synthesis. Chemical Research in Toxicology. 2012; 25: 1893–1901.
[150] Lin CY, Chen JH, Fu RH, Tsai CW. Induction of Pi form of glutathione S-transferase by carnosic acid is mediated through PI3K/Akt/NF-κB pathway and protects against neurotoxicity. Chemical Research in Toxicology. 2014; 27: 1958–1966.
[151] Dringen R, Hirrlinger J. Glutathione pathways in the brain. Biological Chemistry. 2003; 384: 505–516.
[152] Wu CR, Tsai CW, Chang SW, Lin CY, Huang LC, Tsai CW. Carnosic acid protects against 6-hydroxydopamine-induced neurotoxicity in in vivo and in vitro model of Parkinson's disease: involvement of antioxidative enzymes induction. Chemico-biological Interactions. 2015; 225: 40–46.
[153] de Oliveira MR, Ferreira GC, Schuck PF. Protective effect of carnosic acid against paraquat-induced redox impairment and mitochondrial dysfunction in SH-SY5Y cells: Role for PI3K/Akt/Nrf2 pathway. Toxicology in Vitro. 2016; 32: 41–54.
[154] de Oliveira MR, Ferreira GC, Schuck PF, Dal Bosco SM. Role for the PI3K/Akt/Nrf2 signaling pathway in the protective effects of carnosic acid against methylglyoxal-induced neurotoxicity in SH-SY5Y neuroblastoma cells. Chemico-Biological Interactions. 2015; 242: 396–406.
[155] Allaman I, Bélanger M, Magistretti PJ. Methylglyoxal, the dark side of glycolysis. Frontiers in Neuroscience. 2015; 9: 23.
[156] de Oliveira MR, de Souza ICC, Fürstenau CR. Carnosic Acid Induces Anti-Inflammatory Effects in Paraquat-Treated SH-SY5Y Cells Through a Mechanism Involving a Crosstalk Between the Nrf2/HO-1 Axis and NF-κB. Molecular Neurobiology. 2018; 55: 890–897.
[157] Wegiel B, Otterbein LE. Go green: the anti-inflammatory effects of biliverdin reductase. Frontiers in Pharmacology. 2012; 3: 47.
[158] de Oliveira MR, Peres A, Ferreira GC, Schuck PF, Gama CS, Bosco SMD. Carnosic Acid Protects Mitochondria of Human Neuroblastoma SH-SY5Y Cells Exposed to Paraquat Through Activation of the Nrf2/HO-1Axis. Molecular Neurobiology. 2017; 54: 5961–5972.
[159] de Souza ICC, Gobbo RCB, de Almeida FJS, Luckachaki MD, de Oliveira MR. Carnosic acid depends on glutathione to promote mitochondrial protection in methylglyoxal-exposed SH-SY5Y cells. Metabolic Brain Disease. 2021; 36: 471–481.
[160] Lin CY, Tsai CW, Tsai CW. Carnosic acid protects SH-SY5Y cells against 6-hydroxydopamine-induced cell death through upregulation of parkin pathway. Neuropharmacology. 2016; 110: 109–117.
[161] Lin CY, Tsai CW. PINK1/parkin-mediated mitophagy pathway is related to neuroprotection by carnosic acid in SH-SY5Y cells. Food and Chemical Toxicology. 2019; 125: 430–437.
[162] Lin CY, Huang YC, Wu CR, Wu HT, Fu RH, Tsai CW. Carnosic acid attenuated cytochrome c release through the mitochondrial structural protein Mic60 by PINK1 in SH-SY5Y cells. Food and Chemical Toxicology. 2023; 173: 113636.
[163] Lin CY, Tsai CW. Carnosic Acid Attenuates 6-Hydroxydopamine-Induced Neurotoxicity in SH-SY5Y Cells by Inducing Autophagy Through an Enhanced Interaction of Parkin and Beclin1. Molecular Neurobiology. 2017; 54: 2813–2822.
[164] Fu RH, Huang LC, Lin CY, Tsai CW. Modulation of ARTS and XIAP by Parkin Is Associated with Carnosic Acid Protects SH-SY5Y Cells against 6-Hydroxydopamine-Induced Apoptosis. Molecular Neurobiology. 2018; 55: 1786–1794.
[165] Lin CY, Chen WJ, Fu RH, Tsai CW. Upregulation of OPA1 by carnosic acid is mediated through induction of IKKγ ubiquitination by parkin and protects against neurotoxicity. Food and Chemical Toxicology. 2020; 136: 110942.
[166] Lin CY, Huang YN, Fu RH, Liao YH, Kuo TY, Tsai CW. Promotion of mitochondrial biogenesis via the regulation of PARIS and PGC-1α by parkin as a mechanism of neuroprotection by carnosic acid. Phytomedicine. 2021; 80: 153369.
[167] Brasil FB, de Almeida FJS, Luckachaki MD, Dall'Oglio EL, de Oliveira MR. Astaxanthin prevents mitochondrial impairment in the dopaminergic SH-SY5Y cell line exposed to glutamate-mediated excitotoxicity: Role for the Nrf2/HO-1/CO-BR axis. European Journal of Pharmacology. 2021; 908: 174336.
[168] Nishimoto S, Koike S, Inoue N, Suzuki T, Ogasawara Y. Activation of Nrf2 attenuates carbonyl stress induced by methylglyoxal in human neuroblastoma cells: Increase in GSH levels is a critical event for the detoxification mechanism. Biochemical and Biophysical Research Communications. 2017; 483: 874–879.
[169] Mailloux RJ. Protein S-glutathionylation reactions as a global inhibitor of cell metabolism for the desensitization of hydrogen peroxide signals. Redox Biology. 2020; 32: 101472.
[170] Okatsu K, Koyano F, Kimura M, Kosako H, Saeki Y, Tanaka K, *et al*. Phosphorylated ubiquitin chain is the genuine Parkin receptor. The Journal of Cell Biology. 2015; 209: 111–128.
[171] Tanaka K, Suzuki T, Hattori N, Mizuno Y. Ubiquitin, proteasome and parkin. Biochimica et Biophysica Acta. 2004; 1695: 235–247.
[172] Chien WL, Lee TR, Hung SY, Kang KH, Wu RM, Lee MJ, *et al*. Increase of oxidative stress by a novel PINK1 mutation, P209A. Free Radical Biology & Medicine. 2013; 58: 160–169.
[173] van der Laan M, Horvath SE, Pfanner N. Mitochondrial contact site and cristae organizing system. Current Opinion in Cell Biology. 2016; 41: 33–42.
[174] Tsai PI, Lin CH, Hsieh CH, Papakyrikos AM, Kim MJ, Napolioni V, *et al*. PINK1 Phosphorylates MIC60/Mitofilin to Control Structural Plasticity of Mitochondrial Crista Junctions. Molecular Cell. 2018; 69: 744–756.e6.
[175] Akabane S, Uno M, Tani N, Shimazaki S, Ebara N, Kato H, *et al*. PKA Regulates PINK1 Stability and Parkin Recruitment to Damaged Mitochondria through Phosphorylation of MIC60. Molecular Cell. 2016; 62: 371–384.
[176] Tran S, Fairlie WD, Lee EF. BECLIN1: Protein Structure, Function and Regulation. Cells. 2021; 10: 1522.
[177] Wu Y, Li X, Zhu JX, Xie W, Le W, Fan Z, *et al*. Resveratrol-activated AMPK/SIRT1/autophagy in cellular models of Parkinson's disease. Neuro-Signals. 2011; 19: 163–174.
[178] Sun Y, Cai Y, Qian S, Chiou H, Zang QS. Beclin-1 improves mitochondria-associated membranes in the heart during endotoxemia. FASEB BioAdvances. 2021; 3: 123–135.
[179] Raturi A, Simmen T. Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM). Biochimica et Biophysica Acta. 2013; 1833: 213–224.
[180] Patergnani S, Suski JM, Agnoletto C, Bononi A, Bonora M, De Marchi E, *et al*. Calcium signaling around Mitochondria Associated Membranes (MAMs). Cell Communication and Signaling. 2011; 9: 19.
[181] Vance JE. MAM (mitochondria-associated membranes) in mammalian cells: lipids and beyond. Biochimica et Biophysica Acta. 2014; 1841: 595–609.
[182] Kemeny S, Dery D, Loboda Y, Rovner M, Lev T, Zuri D, *et al*. Parkin promotes degradation of the mitochondrial pro-apoptotic ARTS protein. PLoS ONE. 2012; 7: e38837.
[183] Bornstein B, Gottfried Y, Edison N, Shekhtman A, Lev T, Glaser F, *et al*. ARTS binds to a distinct domain in XIAP-BIR3 and promotes apoptosis by a mechanism that is different from other IAP-antagonists. Apoptosis. 2011; 16: 869–881.
[184] Santos D, Cardoso SM. Mitochondrial dynamics and neuronal fate in Parkinson's disease. Mitochondrion. 2012; 12: 428–437.
[185] Gao XY, Yang T, Gu Y, Sun XH. Mitochondrial Dysfunction

in Parkinson's Disease: From Mechanistic Insights to Therapy. Frontiers in Aging Neuroscience. 2022; 14: 885500.
[186] Yu R, Lendahl U, Nistér M, Zhao J. Regulation of Mammalian Mitochondrial Dynamics: Opportunities and Challenges. Frontiers in Endocrinology. 2020; 11: 374.
[187] Laforge M, Rodrigues V, Silvestre R, Gautier C, Weil R, Corti O, *et al*. NF-κB pathway controls mitochondrial dynamics. Cell Death and Differentiation. 2016; 23: 89–98.
[188] Jahani-Asl A, Pilon-Larose K, Xu W, MacLaurin JG, Park DS, McBride HM, *et al*. The mitochondrial inner membrane GTPase, optic atrophy 1 (Opa1), restores mitochondrial morphology and promotes neuronal survival following excitotoxicity. The Journal of Biological Chemistry. 2011; 286: 4772–4782.
[189] Ramonet D, Perier C, Recasens A, Dehay B, Bové J, Costa V, *et al*. Optic atrophy 1 mediates mitochondria remodeling and dopaminergic neurodegeneration linked to complex I deficiency. Cell Death and Differentiation. 2013; 20: 77–85.
[190] Olichon A, Baricault L, Gas N, Guillou E, Valette A, Belenguer P, *et al*. Loss of OPA1 perturbates the mitochondrial inner membrane structure and integrity, leading to cytochrome c release and apoptosis. The Journal of Biological Chemistry. 2003; 278: 7743–7746.
[191] Gureev AP, Shaforostova EA, Popov VN. Regulation of Mitochondrial Biogenesis as a Way for Active Longevity: Interaction Between the Nrf2 and PGC-1α Signaling Pathways. Frontiers in Genetics. 2019; 10: 435.
[192] Shin JH, Ko HS, Kang H, Lee Y, Lee YI, Pletinkova O, *et al*. PARIS (ZNF746) repression of PGC-1α contributes to neurodegeneration in Parkinson's disease. Cell. 2011; 144: 689–702.
[193] Whitaker RM, Corum D, Beeson CC, Schnellmann RG. Mitochondrial Biogenesis as a Pharmacological Target: A New Approach to Acute and Chronic Diseases. Annual Review of Pharmacology and Toxicology. 2016; 56: 229–249.
[194] Posadas SJ, Caz V, Largo C, De la Gándara B, Matallanas B, Reglero G, *et al*. Protective effect of supercritical fluid rosemary extract, Rosmarinus officinalis, on antioxidants of major organs of aged rats. Experimental Gerontology. 2009; 44: 383–389.
[195] Farr SA, Niehoff ML, Ceddia MA, Herrlinger KA, Lewis BJ, Feng S, *et al*. Effect of botanical extracts containing carnosic acid or rosmarinic acid on learning and memory in SAMP8 mice. Physiology & Behavior. 2016; 165: 328–338.
[196] Uruno A, Motohashi H. The Keap1-Nrf2 system as an in vivo sensor for electrophiles. Nitric Oxide: Biology and Chemistry. 2011; 25: 153–160.
[197] Miller DM, Singh IN, Wang JA, Hall ED. Administration of the Nrf2-ARE activators sulforaphane and carnosic acid attenuates 4-hydroxy-2-nonenal-induced mitochondrial dysfunction ex vivo. Free Radical Biology & Medicine. 2013; 57: 1–9.
[198] Miller DM, Singh IN, Wang JA, Hall ED. Nrf2-ARE activator carnosic acid decreases mitochondrial dysfunction, oxidative damage and neuronal cytoskeletal degradation following traumatic brain injury in mice. Experimental Neurology. 2015; 264: 103–110.
[199] Samy DM, Mostafa DK, Saleh SR, Hassaan PS, Zeitoun TM, Ammar GAG, *et al*. Carnosic Acid Mitigates Depression-Like Behavior in Ovariectomized Mice via Activation of Nrf2/HO-1 Pathway. Molecular Neurobiology. 2023; 60: 610–628.
[200] Yao W, Lin S, Su J, Cao Q, Chen Y, Chen J, *et al*. Activation of BDNF by transcription factor Nrf2 contributes to antidepressant-like actions in rodents. Translational Psychiatry. 2021; 11: 140.
[201] AlKahtane AA, Ghanem E, Bungau SG, Alarifi S, Ali D, Al-Basher G, *et al*. Carnosic acid alleviates chlorpyrifos-induced oxidative stress and inflammation in mice cerebral and ocular tissues. Environmental Science and Pollution Research International. 2020; 27: 11663–11670.
[202] Liu Y, Zhang Y, Hu M, Li YH, Cao XH. Carnosic acid alleviates brain injury through NF κB regulated inflammation and Caspase 3 associated apoptosis in high fat induced mouse models. Molecular Medicine Reports. 2019; 20: 495–504.
[203] Maynard ME, Underwood EL, Redell JB, Zhao J, Kobori N, Hood KN, *et al*. Carnosic Acid Improves Outcome after Repetitive Mild Traumatic Brain Injury. Journal of Neurotrauma. 2019; 36: 2147–2152.
[204] Gee LE, Chen N, Ramirez-Zamora A, Shin DS, Pilitsis JG. The effects of subthalamic deep brain stimulation on mechanical and thermal thresholds in 6OHDA-lesioned rats. The European Journal of Neuroscience. 2015; 42: 2061–2069.
[205] Ghasemzadeh Rahbardar M, Hemadeh B, Razavi BM, Eisvand F, Hosseinzadeh H. Effect of carnosic acid on acrylamide induced neurotoxicity: *in vivo* and *in vitro* experiments. Drug and Chemical Toxicology. 2022; 45: 1528–1535.
[206] Babahajian A, Sarveazad A, Golab F, Vahabzadeh G, Alizadeh A, Rasoolijazi H, *et al*. Neuroprotective Effects of Trolox, Human Chorionic Gonadotropin, and Carnosic Acid on Hippocampal Neurodegeneration After Ischemiareperfusion Injury. Current Stem Cell Research & Therapy. 2019; 14: 177–183.
[207] Teng L, Fan L, Peng Y, He X, Chen H, Duan H, *et al*. Carnosic Acid Mitigates Early Brain Injury After Subarachnoid Hemorrhage: Possible Involvement of the SIRT1/p66shc Signaling Pathway. Frontiers in Neuroscience. 2019; 13: 26.