Review

Therapeutic Potential of Caffeic Acid Phenethyl Ester (Cape) and Derivatives With Anti-inflammatory, Antioxidant, and Neuroprotective Activities

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Review

Therapeutic Potential of Caffeic Acid Phenethyl Ester (CAPE) and Derivatives with Anti-Inflammatory, Antioxidant, and Neuroprotective Activities

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Abstract: Neurodegenerative disorders are characterized by a progressive process of degeneration and neuronal death, where oxidative stress and neuroinflammation are key factors that contribute to the progression of these diseases. Therefore, two major pathways involved in these pathologies have been proposed as relevant therapeutic targets: The nuclear transcription factor erythroid 2 (Nrf2), which responds to oxidative stress with cytoprotecting activity and the nuclear factor NF-κB pathway, which is highly related to the neuroinflammatory process by promoting cytokine expression. Caffeic acid phenethyl ester (CAPE) is a phenylpropanoid naturally found in propolis that shows important biological activities, including neuroprotective activity by modulating the Nrf2 and NF-kB pathways, promoting antioxidant enzyme expression and inhibition of proinflammatory cytokine expression. Its simple chemical structure has inspired the synthesis of many derivatives, with aliphatic and/or aromatic moieties, some of which have improved the biological properties. Moreover, new drug delivery systems increase the bioavailability of these compounds in vivo, allowing its transcytosis through the blood-brain barrier, thus protecting brain cells from the increased inflammatory status associated to neurodegenerative and psychiatric disorders. This review summarizes the biosynthesis and chemical synthesis of CAPE derivatives, their miscellaneous activities, and relevant studies (from 2010 to 2023) addressing their neuroprotective activity in vitro and in vivo.

Keywords: caffeic acid phenethyl ester; CAPE derivatives; anti-inflammatory; NF-kB; antioxidant; Nrf2; neuroprotection

1. Introduction

The current demographic shift of modern societies has led to a surge in the prevalence of diseases affecting older adults, including neurodegenerative disorders. Among them, Alzheimer's disease (AD) is the first cause of dementia and a significant global public health problem, estimated to affect about 131.5 million people worldwide, with an annual incidence of 4 to 6 million new cases (Dubois, Villain et al. 2021). On the other hand, the second neurodegenerative disorder is Parkinson's disease (PD), affecting one in every hundred people over 60 years of age. It is estimated that by 2030 there will be 9 million patients with idiopathic PD worldwide (Erkkinen, Kim et al. 2018). Moreover, psychiatric disorders with increasing worldwide prevalence, such as major depressive disorder (MDD), are relevant and potentially modifiable risk factors for dementia. Neurodegenerative disorders are characterized by excessive damage of key brain structures, which is followed by neuronal function loss, structural alterations and decrease of cellular survival (Heppner, Ransohoff et al. 2015). However, despite our increasing knowledge about their pathogenic mechanisms, successful therapeutic approaches to tackle neurodegeneration have remained highly elusive.

A wealth of evidence has supported that oxidative stress and neuroinflammation may have a crucial role in the pathogenesis of neurodegenerative disorders, as they are associated with complex systems that activate programmed cell death cascades (Ebadi, Srinivasan et al. 1996, Markesbery and Carney 1999, Barber and Shaw 2010, Leiva, Martínez-Sanguinetti et al. 2019). Activation of the Nrf2 and NF-kB pathways are expected to have a prominent role in the control of oxidative stress and inflammation. Nuclear erythroid transcription factor 2 (Nrf2) responds to oxidative stress, mediating cytoprotection in mammalian cells by the production of a set of antioxidant genes called phase II genes, which produce phase II proteins associated to ROS stabilization (Cullinan, Gordan et al. 2004, Kobayashi, Kang et al. 2004, Sykiotis and Bohmann 2010). In turn, NF-kB controls pro-inflammatory gene expression he synthesis of cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, IL-6, and IL-8 are directly mediated by NF-kB, as well as the expression of cyclooxygenase-2 (COX2) (Almowallad, Alqahtani et al. 2022).

To date, conventional single drug-based therapeutic approaches for addressing neurodegenerative disorders have not been entirely satisfactory. For instance, in the pharmacological treatment of AD, since the 1990s, only tree cholinesterase inhibitors including galantamine, rivastigmine, and donepezil together with memantine a glutamatergic antagonist are available. Therefore, targeting multiple pharmacological pathways, such as inflammation and oxidative stress, holds great promise for increasing the likelihood of obtaining potent neuroprotective effects. Among the novel and emerging therapeutic approaches, consuming functional foods enriched with natural bioactive molecules has shown great potential as a palliative or preventative measure for the middle-aged population. For instance, propolis is considered a food supplement with relevant therapeutic properties including antidiabetic, anticancer or antibacterial activities. Propolis is a large mixture of polyphenols, essential oils, and waxes produced by plants as resinous secretions. This is collected by honeybees, which use it as a waterproof substance to seal hive cracks or holes (Borba, Wilson et al. 2017). Furthermore, bees use propolis as an antiseptic material to prevent infections due to its powerful antibacterial (Przybyłek and Karpiński 2019) and antiparasitic properties. (Drescher, Klein et al. 2017) The large molecular variety of propolis depends on many factors, such as location, the season, and plants distributed around the hive. (Machado, Silva et al. 2016) Despite this, propolis always shows antioxidant, anti-inflammatory, bactericidal, and antifungal properties, that could be explained due to the high content and variety of flavonoids with a particular chemical composition. (Freires, Queiroz et al. 2016; Pazin, Monaco et al. 2017; and Tiveron, Rosalen et al. 2016)

Caffeic acid phenethyl ester (CAPE) is found mainly in propolis. It is a natural polyphenol from the phenylpropanoid family, formed by the esterification of caffeic acid and phenethyl alcohol (Figure 1) (Metzner, Bekemeier et al. 1979, Romero, Freire et al. 2019) (Kurek-Górecka, Rzepecka-Stojko et al. 2013). This polyphenol is not the major component of propolis, but is one of the most potent antioxidants, given by the caffeic acid moiety which has a higher antioxidant capacity than related phenylpropanoids such as ferulic acid, and p-coumaric acid (Rice-Evans, Miller et al. 1996). The concentration of CAPE in propolis has a large difference, from 0 to 11 mg/g of propolis,

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quantification by high performance liquid chromatography (HPLC) (Gómez-Caravaca, Gómez-Romero et al. 2006) showed, for example, that propolis collected in Mexico (Ures) has a CAPE concentration of 11.4 mg/g (Hernandez, Goycoolea et al. 2007), followed by propolis from Viveros and Valencia (Spain), with 10,047 and 10,399 mg/g respectively (García Argente 2018). On the other hand, the product from Chile showed 5,798 mg/g, while propolis from China had 4.9 mg/g, in contrast to the propolis from Honduras that did not present CAPE (Argente 2019). The same results were obtained from propolis collected in different regions of Turkey (Ozdal, Ceylan et al. 2019).

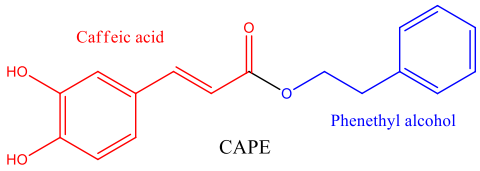

Figure 1. Chemical structure of caffeic acid phenethyl ester (CAPE).

CAPE displays a broad spectrum of beneficial activities, including antitumor activity by inducing apoptosis in many cancer cell lines (Chen, Shiao et al. 2001, Watabe, Hishikawa et al. 2004, Xiang, Wang et al. 2006, Hernandez, Goycoolea et al. 2007). For example, on human leukemia HL-60 cells CAPE inhibits DNA, RNA, and protein synthesis with an IC_{50} of 1.0 μM, 5.0 μM, and 1.5 μM, respectively (Chen, Shao et al. 1996). Moreover, CAPE induces anti-inflammatory and immunomodulatory responses. These activities have been explained by the activation of the erythroid nuclear transcription factor 2 (Nrf2) and the inhibition of the nuclear transcription factor (NF-kB). Thus, CAPE it is available as NF-kB inhibitor for *in vitro* and *in vivo* studies. (Murtaza, Sajjad et al. 2015)

This review provides an in-depth examination of the biosynthesis and chemical synthesis of CAPE derivatives, as well as their various activities, with a particular focus on their effects on the Nrf2 and NF-kB signaling pathways implicated in oxidative and inflammatory damage underlying neurodegeneration.

2. Biosynthesis of CAPE

CAPE is synthesized by the phenylpropanoid pathway, beginning with the amino acids phenylalanine or tyrosine, producing 4-coumaric acid. The biosynthesis of CAPE was reviewed by Song, 2019, and is summarized in Figure 2.

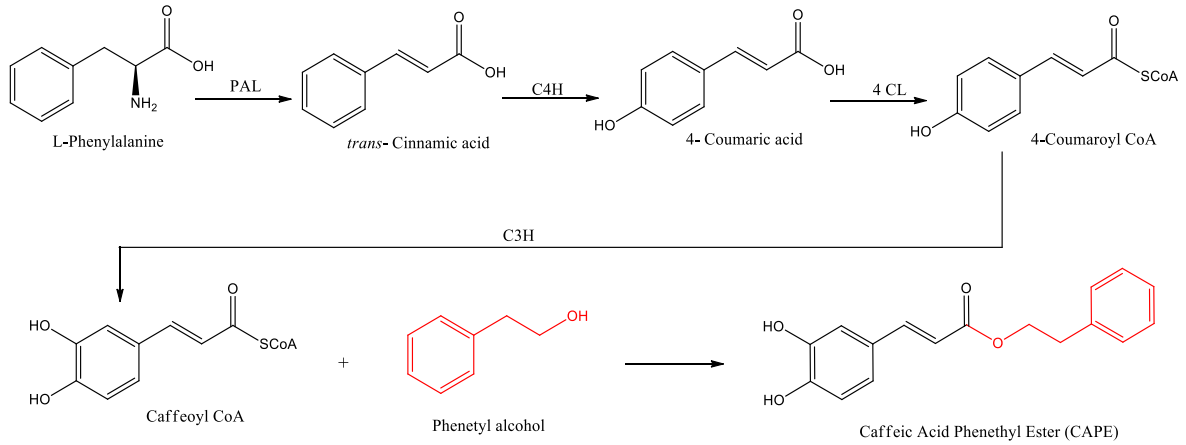

Figure 2. Biosynthetic pathways for the formation of CAPE. The involved enzymes are abbreviated: **PAL** = phenylalanine ammonia lyase; **C4H** = cinnamate 4-hydroxilase; **4CL** = 4-coumaric acid CoA-ligase; **C3H** = p-coumarate 3-hydroxylase.

4-coumaric acid is bound to coenzyme A by 4-coumaric acid CoA-ligase (4CL) enzymatic activity, producing 4-coumaroyl CoA with an activated carbonyl. This product is further meta

hydroxylated by p-coumarate 3-hydroxylase giving the intermediate caffeoyl CoA, which is the precursor of caffeic acid by hydrolysis of CoA, but in the case of biosynthesis of CAPE, the CoA is substituted in one-step with phenethyl alcohol by ester linkage. Phenethyl alcohol is synthesized by two enzymes, an aromatic amino acid decarboxylase such as phenylalanine decarboxylase (PDC) followed by a monoamine oxidase such as phenethylamine oxidase (PEO) (Song, Cho et al. 2019).

The absorption and metabolism of CAPE have not yet been well studied, but it should be similar to caffeic acid or related caffeic acid esters. The absorption and metabolism of the radiolabel [3-^{14}C] caffeic acid have been studied in rats, showing that absorption starts in the stomach after 1 h post-ingestion and is quickly absorbed in the first portion of the intestine, obtaining high concentrations in plasma after 2h, then is excreted mainly by urine as nine derivatives of type sulfonated and glucuronide, with no accumulation in tissues (Omar, Mullen et al. 2012). Caffeic acid esters, such as chlorogenic acid and rosmarinic acid, have shown a similar pattern, being excreted by urine after 4 - 8 h post-ingestion, remaining only 3.3 to 4.0% of total polyphenol content (Omar, Mullen et al. 2012, de Oliveira, Sampaio et al. 2017). Anyway, the pharmacodynamic and pharmacokinetics of murine and human organisms are different. For instance, CAPE shows more stability in human than in rat plasma, where its concentration decreases quickly, producing caffeic acid and by-products. Still, CAPE showed more stability in human plasma, suggesting that CAPE is hydrolyzed by enzymes present in rat plasma but not in human (Celli, Dragani et al. 2007).

3. Biological Properties of CAPE

Numerous studies have evidenced the medicinal properties of propolis, positioning it as a superfood. Many of these beneficial effects are attributable to CAPE, Table 1.

Table 1. Biological properties of CAPE.

Pharmacological effect	Molecular mechanism	Reference
Wound repair	CAPE promotes early inflammatory response (increased NOS2, TNF-α, and NF-κB) associated with a short-term event, leading to fast skin wound healing and inhibition of inflammation. Significant increase in the glutathione (GSH) level, an endogenous antioxidant that plays a key role in cellular defense against oxidative stress. Considerable decrease in malondialdehyde (MDA) and superoxide dismutase activity.	(Serarslan, Altuğ et al. 2007, dos Santos and Monte-Alto-Costa 2013)
Antidiabetic Properties	CAPE is a heme oxygenase 1 (HO-1) inducer, combating the increase of reactive oxygen species (ROS), induced by hyperglycemia. It inhibits the 5-lipoxygenase, alleviating diabetic atherosclerotic symptoms (an important macrovascular complication of diabetes). It restores adipocyte function by increasing adiponectin and PPARγ (their activation leads to improved insulin sensitivity), leading the reduction of proinflammatory factors.	(Choi, Choi et al. 2014, Hassan, El-Bassossy et al. 2014, Pittalà, Salerno et al. 2018, Sorrenti, Raffaele et al. 2019)
Anticancer properties	Inhibition of DNA synthesis, disruption of growth signal transmission, induction of apoptosis through an internal apoptotic pathway and promotion of anti-angiogenic effects. It enhances the anti-cancer effect of first-line chemotherapy drugs, an example being the drug paclitaxel in a rat model of DMBA-induced breast cancer, resulting in lower tumor weights	(Natarajan, Singh et al. 1996, Chen, Chang et al. 2008, Roos, Heiss et al. 2011, Chan, Cheung et

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	compared to those with paclitaxel alone. CAPE protects normal cells against the effects of anticancer drugs by acting as a chemopreventive agent, in the case of the drug irinotecan it protects normal blood, liver and kidney cells without affecting the cytotoxicity of irinotecan in the in vivo model of Ehrlich ascites tumor cells.	al. 2013, Yasui, Nishiyama et al. 2013, Matsunaga, Tsuchimura et al. 2019)
Periodontitis treatment	It improves bone healing, preventing RANKL-induced osteoclastogenesis (causes follicular bone destruction), suggesting its use as a regenerative agent in therapy of bone resorption.	(Kazancioglu, Aksakalli et al. 2015, Kazancioglu, Bereket et al. 2015, Kızıldağ, Arabacı et al. 2019)
Antibacterial activity	High activity against *Mycobactrium tuberculosis, Mycobacterium avium, Streptococcus piogenes Klebsiella pneumoniae* and *Staphylococcus epidermidis*. It presents synergistic activity with many antituberculosis drugs, including rifamycin, streptomycin, isoniazid and antibiotics as gentamicin, tetracycline, chloramphenicol, vancomycin, clindamycin, netilmicin. CAPE Inhibits biofilm formation and lactic acid and extracellular polysaccharide production in *S. mutans.*	(Krol, Scheller et al. 1993, Scheller, Dworniczak et al. 1999, Veloz, Alvear et al. 2019, Niu, Wang et al. 2020)
Antiatherogenic effects	In human platelets CAPE (15 and 25 μM) markedly inhibited platelet aggregation stimulated by collagen (2 μg/ml).	(Chen, Lee et al. 2007)
Estrogenic effects	CAPE is a selective agonist of ER-β estrogen receptors (present in the lungs, blood vessels, brain and bones).	(Jung, Kim et al. 2010)
Hepatoprotective effects	In liver CAPE elevates tissue catalase (CAT) activity and ameliorates ultrastructural changes associated with aging, also gives protection against necrosis, lipid peroxidation, aberrant cell proliferation and p65 activation (decreased number of preneoplastic nodules and reduced incidence of liver tumors). Reduced malondialdehyde (MDA) levels and increased activities of glutathione peroxidase (GPx) together with superoxide dismutase (SOD) in liver tissue.	(Tomur, Kanter et al. 2011, Eşrefoğlu, Iraz et al. 2012)
Lung protection	Effects on pulmonary arterial hypertension and fibrosis by HIF-1α/platelet-derived growth factor (PDGF)-dependent Akt/ERK pathways.	(Cheng, Chi et al. 2019)
Protection of the bone diseases	CAPE induce osteoclast apoptosis, antioxidant effects and modulation of osteoprotegrin signaling pathways, by suppressing NF-κB activity, CAPE significantly inhibits osteoclastogenesis and osteoclast differentiation.	(Ang, Pavlos et al. 2009, Ha, Choi et al. 2009, Zawawi, Perilli

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		et al. 2015, Tolba, El-Serafi et al. 2017)
Alzheimer's disease	CAPE significantly inhibits neuronal apoptosis and neuroinflammation, induces Nrf2 activation, inhibits glycogen synthase kinase 3β in the hippocampus, and improves learning and memory and cognition.	(Morroni, Sita et al. 2018)

4. CAPE Derivatives as Novel Bioactive Compounds

The structure of CAPE has been the inspiration to organic chemists for the development of chemical analogs that improve the activities of the original molecule. The synthesis of CAPE derivatives has been improved over the years, from the initial Fisher esterification catalyzed with acids, giving low yields, to the use of coupling reagents as DCC or by the condensation of acid chlorine derivative, or using microwave assistance. Here we resume some methods that produce acceptable yields and are easy to use (Figure 3).

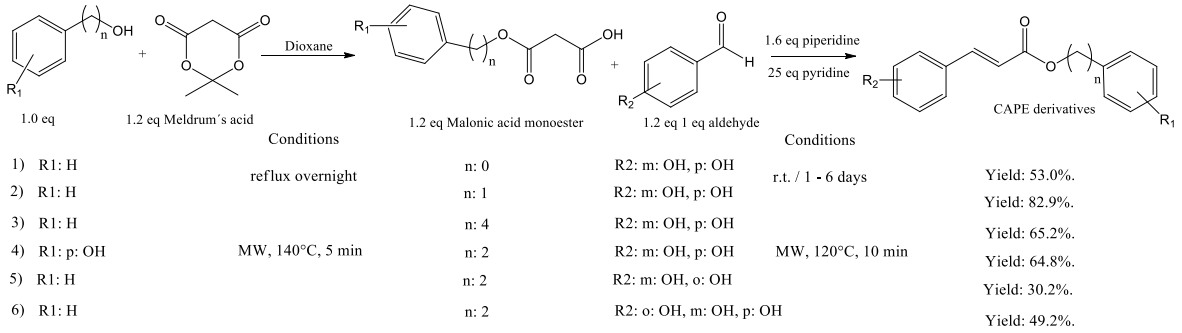
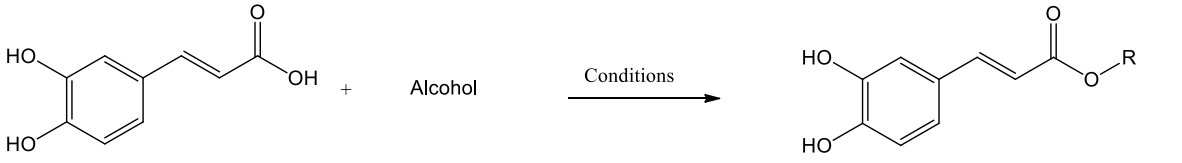


Figure 3. Synthesis of CAPE and derivatives by Knoevenagel condensation using thermal or microwave methods.

Choi et al 2019 produced ester derivatives with the starting alcohol and 1.2 equivalents of Meldrum's acid, by reflux overnight, getting the corresponding malonic acid monoester, then 1 eq. of an aldehyde produces the corresponding CAPE derivative by Knoevenagel condensation, as shown in the Figure 3 entry 1 to 3 (Choi, Villegas et al. 2019). An alternative pathway involves microwave assistance, considerably reducing reaction times but giving moderate yields (entries 4 to 6).

The N,N′-dicyclohexylcarbodiimide (DCC) coupling method was used by Chen et al. in the synthesis of CAPE. Starting with caffeic acid and 5 equivalents of phenethyl alcohol, produced 38% yield of CAPE after purification (Figure 4, entry 1). Nagaoka synthetized twenty CAPE analogues by acyl chlorine condensation, starting with caffeic acid and chlorine thionyl in a dry atmosphere, then the corresponding alcohol was added, producing the corresponding CAPE derivative (Figure 4, entry 2a to 2c). The product 4-phenylbutyl caffeate showed antiproliferative activity with EC_{50} of 20 μM against murine colon 26-L5 carcinoma cells (Nagaoka, Banskota et al. 2002).



1)	5.4 g; 30 mmol)	$HOCH_2CH_2Ph$ 18.0 g, 150 mmol	THF, DCC (60 mmol) r.t. / 8 h stirring	38% yield	Chen et al., 1996
2a)	36 mg, 0.2 mmol	$HOCH_2Ph$	1) $SOCl_2$, dioxane, Ar, 100°C, 3h 2) alcohol, 100°C, 6h	75% yield	Nagaoka et al., 2002
2b)	36 mg, 0.2 mmol	$HO(CH_2)_4Ph$		57% yield	Nagaoka et al., 2002
2c)	36 mg, 0.2 mmol	$HO(CH_2)_{15}CH_3$		35% yield	Nagaoka et al., 2002

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Figure 4. Synthesis of CAPE derivatives by DCC and acyl chlorine condensation.

Different methods have achieved the synthesis of CAPE and its derivatives. However, some routes were not shown here because they present low yields, inconvenient reagents, or burdensome purification procedures, in contrast to the methods summarized in the Figures 3 and 4. CAPE derivatives were studied in vitro and in vivo due to antiviral, and anticancer activities showed. In Figure 5 some of its esters are shown.

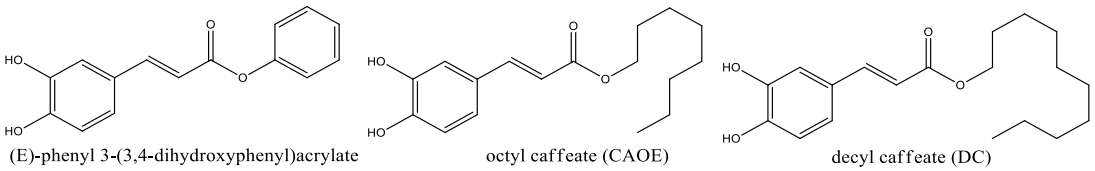

Figure 5. chemical structure of caffeoyl esters derivatives.

Compound (E)-phenyl 3-(3,4-dihydroxyphenyl) acrylate is the best result of a screening of 19 CAPE-derivatives, searching for inhibitors of the enzyme xanthine oxidase (XO). XO inhibition is a therapeutic strategy for treating hyperuricemia, which results in uric acid crystals accumulation in joints, producing gout or inflammatory arthritis. This CAPE-derivative inhibits XO more efficiently than CAPE by π-π interactions with the enzyme. This enzymatic activity, together with the anti-inflammatory capacities of the related molecules, suggests its use in the treatment of hyperuricemia (Choi, Villegas et al. 2019). Moreover, n-Alkyl esters of CAPE have shown antiviral activity against the hepatitis C virus (HCV), with actions depending on and length of the aliphatic chain and substituents in the catechol moiety. Among derivatives with 1, 4, 6, 8, and 10 carbons, octyl caffeate (CAOE, Figure 5) exhibited the highest activity against HCV with an EC_{50} value of 2.7 mM, inhibiting genotype 1b and 2a of the virus, independent of interferon signaling. Moreover, CAOE showed synergistic activity with antivirals such as interferon-alpha 2b, daclatasvir, and VX-222, enhancing their activities. Shen and collaborators evidenced that CAOE is protective against hepato-toxic stimuli and mitochondrial dysfunction induced by tert-butyl hydroperoxide in HepG2 Cells. CAOE showed the best results compared to ten different compounds, including amides and esters of caffeic acid (Shen, Yamashita et al. 2013, Tsai, Yu et al. 2017).

A more extended side chain derivative, with 12 carbons, decyl caffeate (DC, Figure 5) showed inhibition of human colorectal cancer cells HT-29 and HCT-116 cell proliferation, *in vivo* and *in vitro*, on xenograft mouse model, enhancing cell-cycle arrest at the S phase, via blockade of the STAT3 and Akt signaling pathways. Therefore, DC inhibits cell proliferation by inducing autophagy in HCT-116 colon cells (Chen, Kuo et al. 2020).

The replacement of the ester bonding by amide bonding could increase the stability of the molecules regarding acidic pH and esterase activity, increasing their bioavailability. Thus, many caffeoyl amides have been synthesized (Figure 6), and some of them showed neuroprotective and photoprotective effects in cell and animal models.

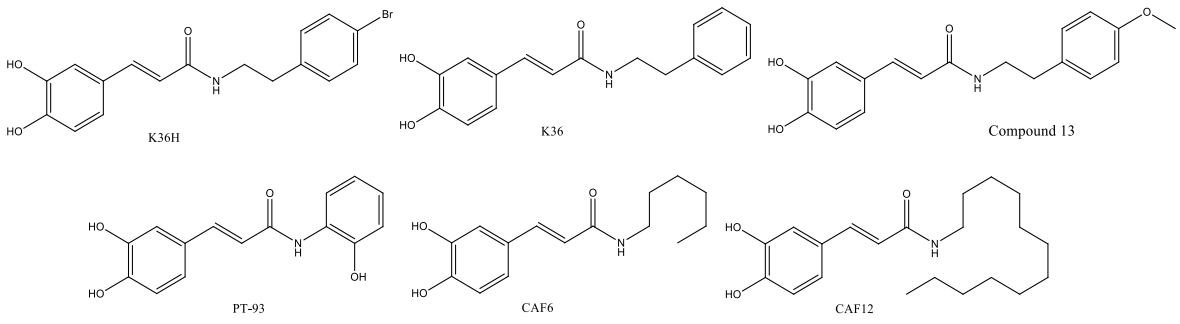

Figure 6. Chemical structure of caffeoyl amides derivatives.

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Compounds K36 and K36H are closely related to CAPE. They change the ester bonding by amide linkage, showing antiphotodamage properties, induced by ultraviolet A (UVA), which is a preponderant factor in skin damage and aging. They were synthesized by condensation of caffeic acid with 2-phenylethanamine or 2-(4-bromophenyl)ethanamine producing K36 or K36H, respectively. K36 reduces ROS levels via nuclear factor erythroid 2-related factor 2 (Nrf2), with the consequent antioxidant response mechanisms, such as the expression of the antioxidant enzyme heme oxygenase-1 (HO-1), together with the inhibition of metalloproteinase (MMP)-1, MMP-2, IL-6, and PGE2. K36, as well as CAPE, reduces iNOS and COX-2 expression by inhibition of NF-κB (Kuo, Chen et al. 2015). Moreover, K36 reduces the activation of extracellular-signal-regulated kinase (ERK) and c-Jun N-terminal kinases (JNK), as well as 8-hydroxy-20-deoxyguanosine (8-OHdG) levels (Chu, Wu et al. 2019). The p-bromine derivative K36H displays similar behavior as K36, reducing ROS generation, PGE2, NO formation, DNA damage, overexpression of matrix metalloproteinase (MMP)-1 and MMP-2, as well as the expression of Bax and caspase-3, in line with photoprotective properties, and suggesting that K36 and K36H could be used as active components of new photoprotector products (Kuo, Chiang et al. 2020).

The long-chain amides, CAPE dodecyl amide (CAF12) together with CAPE hexyl amide (CAF6), showed neuroprotective effects in an animal model of Diabetic Retinopathy by ameliorating oxidative stress via increasing antioxidant enzyme retinal superoxide dismutase (SOD). Moreover, treatment with CAF12 or CAF6 decreased iPF2α levels and AKT phosphorylation in diabetic rats, with the promotion of survival and growth of retinal neurons. This resulted in the prevention of negative morphological changes in the retina of diabetic rat models treated with streptozotocin (a diabetic inductor drug) compared with rats treated with CAF12 or CAF6 intravitreally for two weeks (Fathalipour, Eghtedari et al. 2019). Moreover, prior studies with aliphatic amides of caffeic acid with C3, C4, C6, C8, and C12 were evaluated on AD models, showing that CAF12 increases PC12 pheochromocytoma cell survival in serum-deprived conditions, enhancing the nerve growth factor (NGF) effect, and inducing neurite outgrowth via the ERK1/2 and AKT pathways, by activation of PI3K. Moosavi and colleagues suggested that these amides could have neuroprotective properties (Moosavi, Hosseini et al. 2017). A CAF12 and CAF6-related amide, denominated compound 13, with a p-methoxy group incorporated into the phenyl moiety, revealed a potent antioxidant and neuroprotection activity with better results compared to other 20 related compounds evaluated (Chai, Zhao et al. 2018). Compound 13 induced memory loss impairment in a transgenic *Drosophila* model, as well as inhibitory or disaggregation activity on Aβ peptides in Alzheimer's disease (AD) models (Chai, Zhao et al. 2018). Although these amides are structurally related to the esters shown in Figure 3, and possess antiviral and anticancer activities, they have yet not been studied adequately in these disease models. Only a molecule with a shorter side chain, the amide PT-93, was studied in glioblastoma multiforme (GBM) such as T98G, U87, U251 and HT22 cell lines. The compound showed antiproliferative activities in these models by MMP-2 and MMP-9 inhibition (Li, Tu et al. 2017).

Amides and esters presented here are the result of inducing chemical variations in the phenethyl moiety of CAPE, producing a variety of bioactive compounds. Moreover, Wan, et al 2019 improved the solubility and absorption of CAPE with the synthesis pf the 4-O-glucoside of CAPE, called FA-97, by coupling of CAPE to an acetyl protected brominated D-glucose, according to the Figure 7 (Wan, Wang et al. 2019).

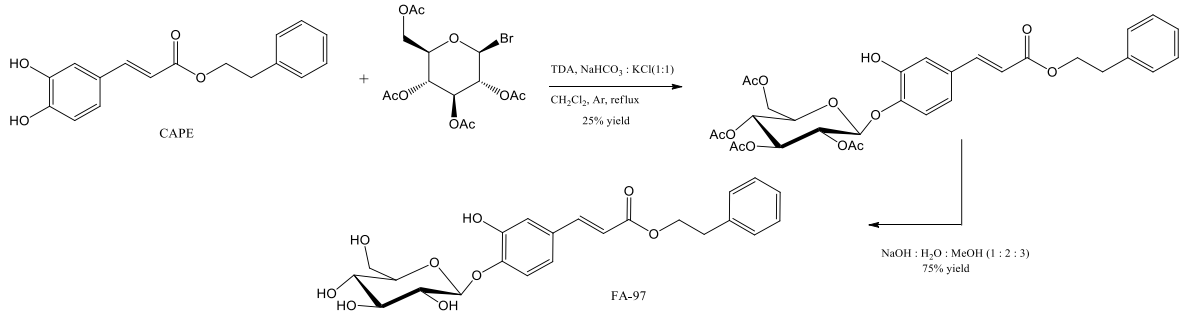

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Figure 7. Synthesis of caffeic acid phenethyl ester 4-O-glucoside, or FA-97 (Wan, Wang et al. 2019).

FA-97 enhances antioxidant and scavenger properties in neuronal cell cultures by directly inhibiting ROS production while also activating Nrf2, which produces an antioxidant cell response. In SH-SY5Y human neuroblastoma and PC12 rat pheochromocytoma cells, FA-97 attenuated H_2O_2-induced apoptosis by decreasing ROS and malondialdehyde (MDA) levels, also induces cellular superoxide (SOD) and glutathione (GSH) dismutase, quinone oxidoreductase 1(NQO1) and HO-1, by activation of the Nrf2 pathway. On AD models, FA-97 reduced learning and memory impairments in mice via reducing neuronal apoptosis (Wan, Wang et al. 2019). On another hand, FA-97 showed to be a potential drug against inflammatory bowel disease (IBD), which is an inflammatory intestinal disorder. In fact, on a mouse model of colitis employing dextran sodium sulfate (DSS) to induce epithelial damage, FA-97 reduced body weight loss, as well as colon damage and length shortening, via activation of HO-1/Nrf2, showing the excellent properties of this compound in gastrointestinal affections (Mei, Wang et al. 2019).

5. CAPE Inhibits Oxidative Stress by Modulation of the Nrf2 Pathway

The astonishing metabolic activity of the brain renders it particularly susceptible to oxidative damage. Indeed, despite the brain accounts for only 2% of our body weight, it consumes 20% of the whole body's oxygen supply. This puts neuronal cells at risk of generating an excess of free radicals that can overwhelm the available antioxidant systems, leading to oxidative stress (OS). Furthermore, OS is associated with the development and progression of different neurodegenerative disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) (Sies 1986, Halliwell and Gutteridge 2015). Interestingly, transcription factors such as Nrf2 govern the homeostatic oxidative responses, highlighting its potential as a therapeutic target for counterbalancing oxidative damage during neurodegenerative disorders. Indeed, Nrf2 has been shown to upregulate the expression of various antioxidant enzymes, which can be further stimulated with CAPE. Next, we will briefly provide mechanistic insights regarding the effects of chemical modifications during OS associated with neurodegeneration. Further, we will examine evidence supporting the potential of CAPE for positively impacting Nrf2 signaling during neurodegeneration (Dawson and Dawson 1996, Halliwell 2001).

Metabolic activity is a major source of OS in the cells. As such, mitochondria are the major sources of cellular ATP and the main generators of Reactive Oxygen Species (ROS) and OS inside the cells. Indeed around 2% of the oxygen consumed by cells is converted into ROS during oxidative phosphorylation in the mitochondria. Different mechanisms may account for the tight association of OS with neurodegeneration. For instance, mitochondrial exposure to ROS may cause damage of its DNA (DNAmit), including DNA fragmentation, chromatid exchange, translocations and consequent mutations. Altered DNAmit impacts on the expression and abnormal activity of proteins involved in ATP synthesis by uncoupling the electronic transport chain from oxidative phosphorylation (Dawson and Dawson 1996, Halliwell 2001), decreasing ATP production and therefore the activity of the plasma membrane ATPase. Decreased activity of the sodium/potassium transporter (Na/K-ATPase) leads to neuronal membrane depolarization, thus generating an increase in cellular excitation, and facilitating the opening of voltage-sensitive Ca^{2+} channels. In turn, increased glutamate release (and decreased uptake by the glutamate transporter) leads to activation of the N-methyl-D-aspartate (NMDA)- glutamate receptor, thus contributing to increased Ca^{2+} influx (Hammer, Parker et al. 1993, Halliwell 2001). Moreover, calcium as activates several Ca^{2+}-dependent signaling pathways, such as nitric oxide synthases (NOS) leading to an excess in the production of nitric oxide (NO) which also generates free radicals causing oxidative damage (Sapolsky 1996, Egan, Kojima et al. 2003, Ceccatelli, Tamm et al. 2007, Liu, Lee et al. 2009).

Oxidative damage is manifested as covalent modifications of different subcellular structures and compartments. Accordingly, ROS can oxidate macromolecules of various nature, including lipids, proteins and nucleic acids, modifying their structure and function (Anand, Kunnumakkara et al. 2007; Tonda-Turo, Origlia et al. 2018). As such, lipid damage by peroxidation is associated to early

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and late markers, including as hexanonyl-lysine (HEL), acrolein-lysine (ACR), malondialdehyde, F2-isoprostanes and 4-hydroxynonenal (4-HNE) adducts. In turn, oxidative damage of DNA and RNA is reflected in markers such as 8-hydroxy-2-deoxyguanosine (8-OHdG) and 8-hydroxyguanine (8-oxo-dGo) respectively, which have a high mutagenic power (Hayashi 2009, Halliwell and Gutteridge 2015). In the case of AD and HD, an increase of oxidized molecules such as proteins, lipids and nucleic acids is generated by the presence of 3-nitrotyrosine and 3,3'-dithyrosine in the hippocampus, neocortex and cerebrospinal ventricular fluid of patients with the condition (Marlatt, Lucassen et al. 2008). In addition, nuclear and mitochondrial DNA modification and lipoperoxidation are associated with increased 8-OHdG, acrolein, malondialdehyde, 4-HNE and F2-isoprostanes (Kamat, Gadal et al. 2008). It has been shown that there is a relationship between increased 4-HNE in cultured hippocampal cells and altered Na+/K+ ATPase function (Tabrizi, Cleeter et al. 1999, Sonnen, Breitner et al. 2008, Zhou, Huang et al. 2008). PD patients show a reduced activity of antioxidant enzymes such as catalase and glutathione peroxidase and the most affected dopaminergic brain region presents a decrease in reduced glutathione (GSH) levels, so the ratio between GSH/GSSG (oxidized glutathione) decreases during neuronal degeneration, which favors the formation of free radicals (Borrás, Sastre et al. 2003, Zhou, Huang et al. 2008). Also, as in amyotrophic lateral sclerosis (ALS), there is a decrease in the activity of Complex I of the mitochondrial electron transport chain accompanied by up to 7-fold increase in O_2, H_2O_2 and NO production (Barber and Shaw 2010).

The transcription factor Nrf2 is the intrinsic controller of cellular redox homeostasis and regulates the expression of a set of antioxidant genes called phase II genes, which produce phase II proteins associated to ROS stabilization. Under normal redox conditions, Nrf2 associates with the Keap1 protein, forming the Keap1-Nrf2 complex that is retained in the cytosol (cytoskeleton) (Cullinan, Gordan et al. 2004, Kobayashi, Kang et al. 2004, Sykiotis and Bohmann 2010). On the other hand, in an oxidizing environment, phosphatidylinositol 3-kinase (PI3K) produces depolymerization of actin microfilaments, realizing the Nrf2 from the cytoskeleton (Lee and Johnson 2004), facilitating its nuclear translocation and allowing its binding to a specific DNA sequence known as ARE (Antioxidant Response Element), producing enzymes of phase II as NQO1 (NADPH Quinone Dehydrogenase 1), glutathione S-transferase (GST) that conjugates ROS and hydrophobic electrophiles to glutathione, UDP-glucuronosyl transferases that conjugate xenobiotics to glucuronic acid for excretion, ferritin for iron storage, epoxide hydrolase that inactivates epoxides, and γ-glutamyl cysteine synthetase (γ-GCS) involved in GSH synthesis, heme oxygenase 1 (HO-1) that catabolizes heme groups, as well as exerting antioxidant and anti-inflammatory actions (Itoh, Tong et al. 2004, Loboda, Damulewicz et al. 2016).

CAPE shows potent antioxidant activity, reducing the formation of the superoxide anion produced during the autoxidation of β-mercaptoethanol and quenching the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. It also inhibited the activity of xanthine oxidase (XO). In addition, CAPE exhibits potent cytoprotective and antigenotoxic antilipoperoxidative potential against oxidative damage (Wang, Chen et al. 2008, Chen, Huang et al. 2009, Wang, Stavchansky et al. 2010). CAPE activates the expression of the antioxidant HO-1 and protects the kidneys against aging-related oxidative injury in rats, by reducing malondialdehyde (MDA) levels with a simultaneous elevation of superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione in the renal tissues of old rats (Chen, Tran et al. 2012, Eşrefoğlu, Iraz et al. 2012). CAPE possesses a marked iron-binding capacity, therefore, interferes with the formation of the ferrous-ferrozin complex (Göçer and Gülçin 2011). Sun et al. demonstrated that CAPE at micromolar concentrations in a concentration-dependent manner is capable of increasing Nrf2 by inhibiting ubiquitination and promoting nuclear translocation of Nrf2 with subsequent HO-1 expression (Kim, Kim et al. 2013). Furthermore, they demonstrated that CAPE regulates NF-κB signaling and the inflammatory response through the Nrf2/HO-1 signaling pathway in IL-1β-stimulated chondrocytes (Sun, Xie et al. 2022).

CAPE attenuates oxidative stress and neurotoxicity, by modulating MAPK and Akt/glycogen synthase kinase 3β (GSK3β) signaling pathways, reducing hippocampal cells dead in an AD mouse model (Huang Y), thus is particularly beneficial in AD treatment due to Alzheimer's disease is closely related to toxicity in hippocampus. Moreover, it was found to protect transient ischemic injury and

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prevent neonatal encephalopathy, by blocking NF-κB-mediated neuronal inflammation, evoking antioxidant effects and mitochondrial apoptosis (Khan, Elango et al. 2007).

CAPE may be able to ameliorate Parkinson's disease, in PD murine models, attenuating dopaminergic loss and decreasing behavioral abnormality through oxidative stress and reducing neuroinflammation, causing a protective effect on nigral dopaminergic neurons by HO-1-dependent MAPK signaling (Fontanilla, Ma et al. 2011, Kurauchi, Hisatsune et al. 2012).

In an acute brain damage model of stroke, CAPE decreased MDA levels, prevented lipid peroxidation due to ROS scavenging, and increased GSH levels and NO in brain tissue (Aladag, Turkoz et al. 2006). When CAPE was compared with alpha-tocopherol, a potent free radical scavenger molecule (vitamin e) on ischemia-reperfusion brain injury, CAPE proved to be better than vitamin e in reducing the brain level of MDA, a marker of oxidative stress (Irmak, Fadillioglu et al. 2003). Moreover, CAPE is highly effective in scavenging free radicals, even more than their structurally related ferulic acid and ethyl ferulate, as well as in blocking the enhanced release of cardiolipin and cytochrome c, therefore it protects from functional alterations in isolated mouse brain mitochondria challenged by anoxia/reoxygenation (Feng, Lu et al. 2008). This compound further exhibits an effect on isolated brain mitochondria by directly inhibiting Ca^{2+}-induced cytochrome c release, caspase-3, caspase-1, expression of inducible nitric oxide synthase by hypoxia-mediated, *in vivo*. Moreover, CAPE potently blocked neurotoxicity *in vitro* due to nitric oxide-induced (Wei, Zhao et al. 2004). The mechanisms of action of CAPE on oxidative stress and activation of the Nrf2 pathway are summarized in Figure 8.

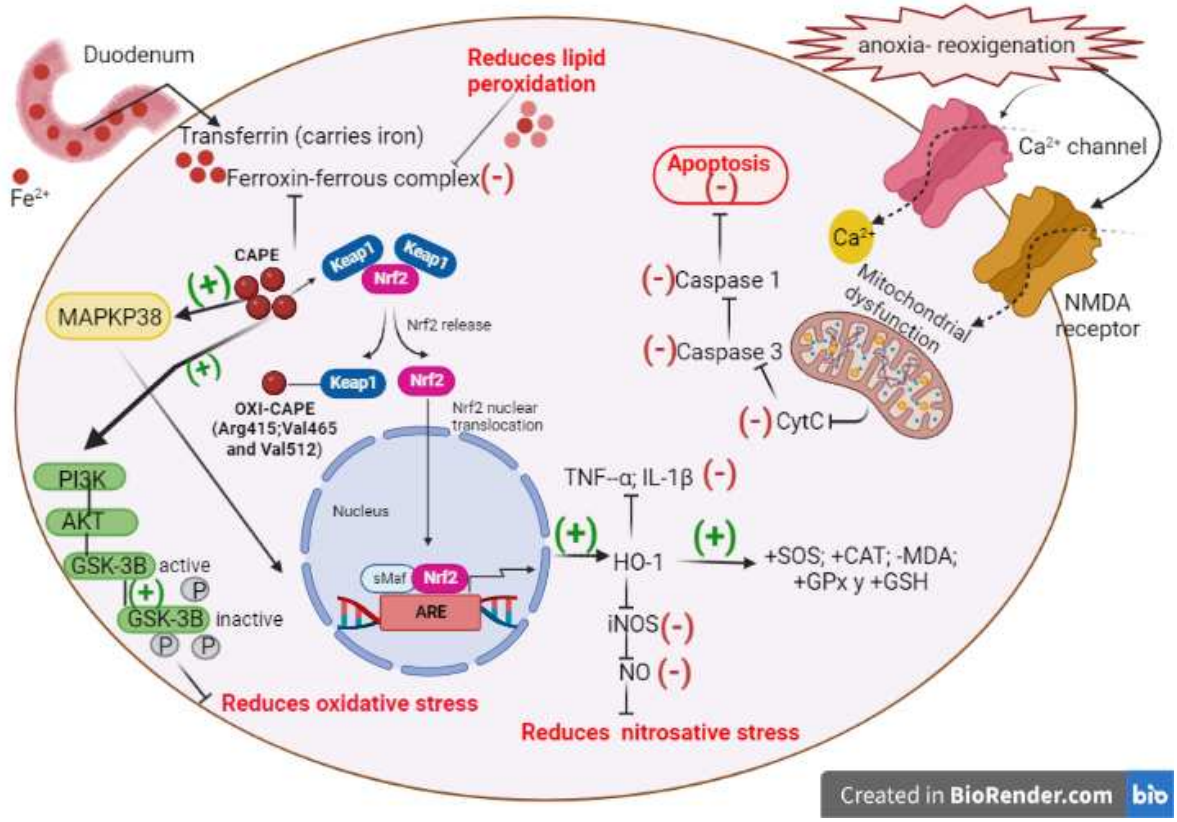

Figure 8. CAPE action mechanisms on oxidative stress and activation of the Nrf2 pathway. (-) routes that inactivate. (+) activating routes.

6. CAPE Inhibits Neuroinflammation by Modulation of the NF-κB Pathway

Cumulating evidence supports that the transcription factor NF-κB plays a critical role in regulating the immune response and inflammation implicated in the pathogenesis of neurodegenerative disorders. The inflammatory response involves the activation of the innate and adaptive immune system, leading to the release of proinflammatory and anti-inflammatory cytokines, which play a crucial role in inflammation resolution. These systemic inflammatory

responses are also manifested inside the brain's parenchyma, which is known as neuroinflammation. Furthermore, there is a particular inflammatory signature in the bloodstream and brain of patients with different neurodegenerative disorders, including AD, PD, ALS, and FTD. Interestingly, CAPE may be a relevant therapeutic agent for controlling NF-κB signaling and reducing neuroinflammation in these disorders.

NF-kB activation can be mediated through three pathways: canonical (or classical), the non-canonical (or alternative) and a non-enzymatic regulatory component. In the canonical pathway, NF-kB is activated in response to viral and microbial infections or exposure to pro-inflammatory cytokines. This occurs with the participation of RelA, and c-Rel dimers together with P50. After activation, the IKKB subunit phosphorylates IkB at serine residues, causing its ubiquitin-dependent degradation via proteasome, translocating NF-kB (p65/p50) to the nucleus to act as a transcription factor for target gene (Mincheva, Garcera et al. 2011). On the other hand, the alternative pathway is activated via members of the TNF-α family, such as lymphotoxin B and BAFF; CK2, independent of the IKK complex (IKKα and IKKβ). The pathway mainly activates IKKa, which is responsible for the phosphorylation of p100, causing proteolysis to give an active form, which is p52. The dimers can then be translocated into the nucleus, acting as transcription factors (Demchenko, Glebov et al. 2010).

Most of the genes under NF-κB transcriptional control are involved in immune system signaling and inflammatory responses. In fact, transcriptional control of cytokine expression by NF-κB is probably one of the most important factors when assessing the pathogenic role of NF-κB in some diseases. Some of these cytokines include TNFα, IL-1α/β, IL-2, 3, 6, 12, GM-CSF, M-CSF and G-CSF, the chemokines MCP-1, KC, MIP-1 and CCL together with adhesion molecules ICAM-1, E-selectin and VCAM-1, which enable the recruitment and binding of immune system cells to sites where the inflammatory process develops (Lai, Liu et al. 2017). NF-κB prevents apoptosis in several inflammatory cells, including macrophages, dendritic cells, T and B-lymphocytes, and neutrophils, and promotes survival of several malignant tumors, especially of lymphomas. In contrast, the inflammatory response can induce apoptotic cell death. This inflammatory cell death response is initiated by the production of cell death receptors (Fas and FasL) and intracellular apoptosis-inducing proteins. This apoptotic death is further assisted by activated immune cells, which secrete granzyme, perforin and nitric oxide, all apoptosis-inducing factors regulated by NF-κB (Lai, Liu et al. 2017).

The mammalian NF-κB family consists of five subfamilies: NF-κB1 (p105 and p50), NF-κB2 (p100 and p52), RelA (p65), RelB and c-Rel. In most cell types, NF-kB dimers are predominantly cytoplasmic because they are in constant interaction with NF-kB inhibitors (IkBα, IkBβ, IkBε and Bcl-3) remaining transcriptionally inactive (Karin and Ben-Neriah 2000). IkBα, IkBβ, IkBε interact with NF-kB dimers and are responsible for their retention in the cytoplasm. These NF-kB inhibitors contain conserved serine residues that can be phosphorylated by IkB kinases (or IKKs), to be subsequently degraded by the proteasome and finally generate the translocation of NF-kB dimers to the nucleus (Oeckinghaus and Ghosh 2009, Sun, Xie et al. 2022).

In 1996 for the first time, the molecular mechanisms by which caffeic acid derivatives prevent NF-kB activation were analyzed. Initially, it was shown that activation of NF-kB by tumor necrosis factor (TNF) is completely blocked by CAPE in a dose- and time-dependent manner. In addition to TNF, CAPE also inhibited NF-kB activation induced by other inflammatory agents, including phorbol ester, ceramide, hydrogen peroxide, and okadaic acid. However, reducing agents reverse inhibitory effects exerted by CAPE, suggesting a role for critical sulfhydryl groups in NF-kB activation (Natarajan, Singh et al. 1996). CAPE prevents translocation of the p65 subunit of NF-kB to the nucleus but has no significant effect on TNF-induced degradation of IκBα; however, it delays IκBα resynthesis. Therefore, these data suggest that CAPE prevented NF-κB activation by inhibiting p65 translocation and IκBα degradation in human chondrocytes (Sun, Xie et al. 2022).

The effect of CAPE on the inhibition of NF-kB binding to is target-DNA sites, as well as the binding to other transcription factors, including AP-1, Oct-1, and TFIID, has been found to be unaffected by CAPE (Natarajan, Singh et al. 1996). When several synthetic structural analogs of CAPE were tested, it was found that a bicyclic, rotationally restricted, 5,6-dihydroxy form exhibits high inhibitory activity, whereas the 6,7-dihydroxy variant is less active. In this sense, other investigations

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in which caffeic acid phenethyl ester (CAPE) and 3,4-dihydroxybenzalacetone (DBL), both phenylpropanoid derivatives containing catechol with and presenting diverse bioactivities, have been evaluated. It was determined that the proinflammatory effects on NO synthase expression was strongly suppressed by CAPE and, to a lesser extent, by DBL and caffeic acid ethyl ester. Thus, the induction of genes downstream of LPS-activated NF-κB, such as NO synthase, IL-1β and IL6, and translocation of NF-κB p65 to the nucleus were reduced, to a greater extent by CAPE than by DBL. Interestingly, phosphorylation of p65 was reduced by both compounds, especially by CAPE, even when the levels of IκB were not altered. One explanation for the action of these compounds is related to the effect of CAPE and DBL on the thiol groups of p65 which were modified by these molecules, however the inhibitory effects on p65 phosphorylation and nitrite production were reversed by pretreatment with thiol-containing reagents, suggesting that CAPE has strong inhibitory effects on NF-κB activation due to modification of thiol groups and phosphorylation of p65 (Natarajan, Singh et al. 1996).

In activated astroglia cells CAPE pretreatment abrogated TNF-α-induced expression of chemokine (CC motif) ligand 2 (CCL-2) and intercellular adhesion molecule 1 (ICAM-1) through inhibition of NF-κB activation in a cell-specific manner (Choi and Choi 2008). In another study CAPE was found to mediate up-regulation of Abcd2 expression and peroxisomal β-oxidation, this causes a decrease in very long chain fatty acid (VLCFA) levels in ABCD1-deficient U87 cells; VLCFA accumulation is characteristic of a neuroinflammatory disease associated with demyelination of the cerebral white matter known as: X-linked adrenoleukodystrophy (X-ALD). CAPE reduced NF-κB activation, iNOS expression and inflammatory cytokines in primary cultures of astrocytes derived from ABCD1 / ABCD2 silenced mice (Singh, Khan et al. 2013).

Microglial activation promotes to mediate inflammatory processes which are crucial in the development of neurodegenerative disorders. CAPE showed to inhibit cyclooxygenase-2 (COX-2) and subsequent NO production in both *in vitro* and *in vivo* models of microglial activation, mediated by α-adenosine monophosphate-activated protein kinase 5′ (AMPK), erythropoietin (EPO) and HO-1 (Tsai, Yu et al. 2017). It is known that in the case of Amyotrophic Lateral Sclerosis the mutation of superoxide dismutase (SOD) is highly correlated with its pathogenesis. A study using murine model showed that CAPE prolongs the survival of mice expressing mutant SOD1 (G93A) by attenuating neuroinflammation and motor neuron cell death as consequence of p38 phosphorylation reduction (Fontanilla, Wei et al. 2012). CAPE may exert its anti-inflammatory effects by inhibiting ROS production at the transcriptional level, through suppressing NF-κB activation and by directly inhibiting the catalytic activity of iNOS (Ilhan et al. 2004). Supposed mechanisms of CAPE actions through the NF-kB pathway are summarized in Figure 9.

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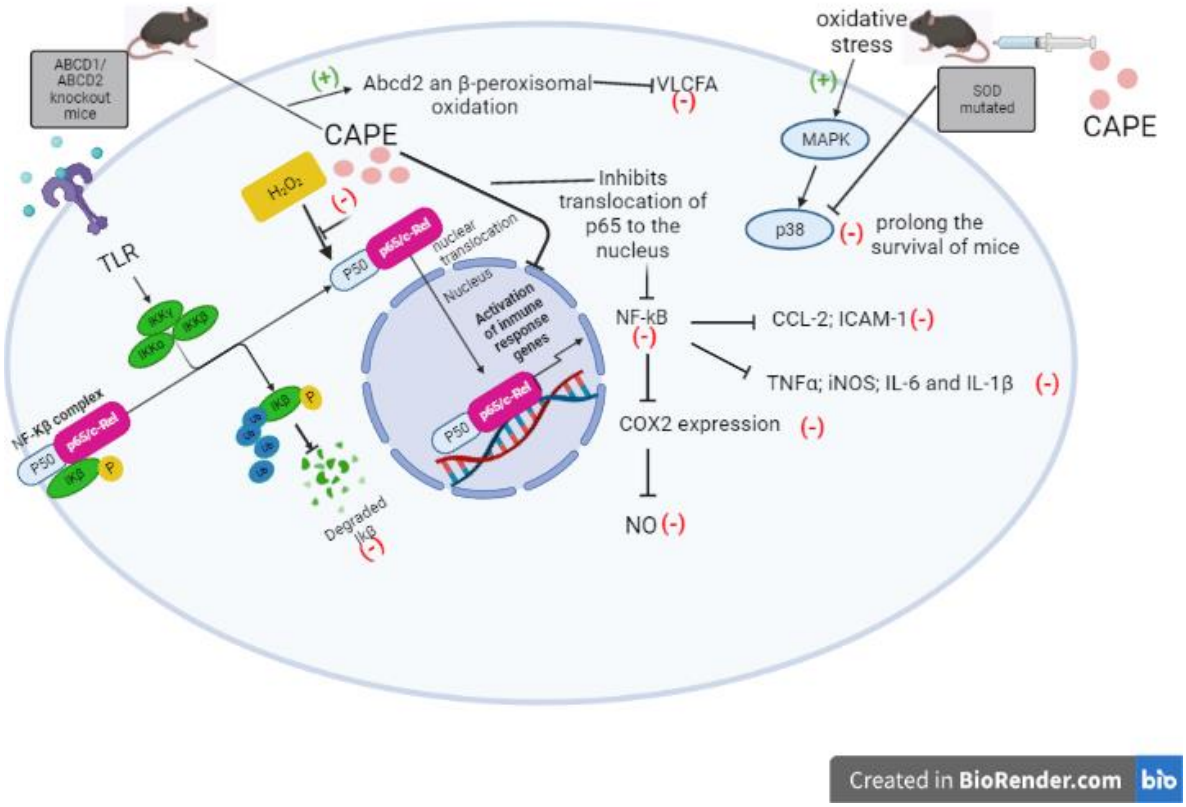

Figure 9. Mechanism of action of CAPE against neuroinflammation and NF-κB pathway activation. (-) routes that inactivate. (+) activating routes.

7. Delivery Systems for Novel CAPE Formulations

The low solubility of CAPE and therefore its low bioavailability, limits its therapeutical use. These problems can be overcome by packing the drug in nanoparticles o vesicles, which provide better therapeutic effects at lower dose. Table 3 summarizes the main delivery systems that have been used with the aim of enhancing the pharmacological effects of CAPE.

Table 3. Bioactive formulation of CAPE.

Formulation	Target	Results	Reference
Poly(lactic-co-glycolic acid) (PLGA) co-loaded nanoparticles (QuCaNP) quercetin and CAPE.	Improving anticancer efficacy in HT-29 human colorectal carcinoma.	Increased caspase-3 (2.38-fold) and caspase-9 (2-fold) mRNA levels and expressions of key proteins in the intrinsic apoptosis pathway in HT-29 cells.	(Dilsu and Erdemir 2021)
Copolymer: polyglycerol and poly(allyl glycidyl ether) (C12-PAGE-PG), loaded with CAPE	To evaluate the *in vitro* and *in vivo* safety of a CAPE-loaded micellar system as a drug delivery platform on HepG2 cells.	Empty micelles loaded with CAPE showed no cytotoxic effects and retained the cytotoxic activity of CAPE loaded in the micelles, making it a good strategy to use this hydrophobic compound and improve the effectiveness of the treatments.	(Yordanov et al. 2020)
NiO nanoparticles and MnO_2 /NiO nanocomposites with	Evaluate its capacity as an anchoring method for drug carriers.	The drug loading time was 100 min and drug release in 1-10 h with 20-80 % drug release.	(Gupta, Fakhri et al. 2017)

guanidine and CAPE as a carrier			
Hyaluronic acid (HA) conjugated with phenylboronic acid pinacol ester (PBPE) with radiosensitive delivery of CAPE	Manufacture radiosensitive delivery of CAPE for application in radioprotection.	Prevention of radiation-induced apoptosis and intracellular ROS accumulation, with increased survivability of mice against radiation-induced death.	(Choi, Lee et al. 2021)
Methoxy poly(ethylene glycol)-*b* -poly(ε-caprolactone) copolymer nanoparticles (CE) with CAPE.	Study antitumor activity against lung metastasis by CT26 colon carcinoma cells.	Superior anti-metastatic efficacy against the tumor than CAPE itself.	(Lee, Jeong et al. 2015)
Caffeic acid phenethyl ester-morphthalin antibody nanoparticles	Generate a potent anticancer drug by recruiting an anti-mortalin antibody (hsp70 chaperone that is enriched on the cancer cell surface).	Enhanced growth arrest/apoptosis of cancer cells through down-regulation of cyclin D1-CDK4, phospho-Rb, PARP-1 and the anti-apoptotic protein Bcl2. Significantly increased expression of p53, p21 WAF1, and caspase cleavage. Significantly improved down-regulation of proteins involved in cell migration	(Wang, Bhargava et al. 2020)
Folic acid-conjugated PLGA nanoparticles.	Improve cytotoxicity, solubility, and achieve sustained release of CAPE.	It showed enhanced cytotoxicity *in vivo* and *in vitro*, causing a decrease in cell proliferation by 46%.	(Kapare, Lohidasan et al. 2021)
Stimuli-responsive liposomal nanocarrier loaded with CAPE modified with ac peptide (RGDyK)	Attack ischemic lesions and remodel neurovascular units (NVU) to reduce the progression of brain injury.	Drugs release in response to pathological signaling stimuli, localization of cerebral ischemia-reperfusion injury and remodeling of neurovascular units by reducing neuronal apoptosis, regulating microglia polarization and repair of vascular endothelial cells.	(Lu, Li et al. 2022)

8. Conclusions

Nowadays neurodegenerative diseases are a global concern with dramatically fast increasing and treatments still been palliatives. In this context functional food could be an alternative in the prevention or deceleration of neurodegenerative problems. It is interesting for the future treatment of AD that propolis has shown to potentiate the neuroprotective effect of memantine in preclinical models of AD (Moriguchi et al. 2022). This evidence supports a new potential avenue of investigation of a combination therapy of CAPE with other drugs to modify the evolution of AD. CAPE has a wide range of pharmacological activities, including neuroprotective effects by inhibition of NF-κB pathways and evoking the intrinsic cell protection by increasing the Nrf-2 pathway. The main objective of this review is to provide a comprehensive overview of the therapeutical potential of CAPE in the treatment of neurodegenerative disorders reviewing the published data about its neuroprotective effects in the last decade. We also shed light on its molecular mechanisms of action

and summarize the structure-activity relationship of synthetic derivatives of CAPE, which will be useful for the further design and development of better derivatives.

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