

## Therapeutic Potential of NRF2 pathway in Neurological Disorder

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### Abstract

Oxidative stress plays a major role in the cause of many diseases including neurological disorders such as Alzheimer's, and Parkinson's to maintain homeostasis between free radicals and antioxidants. Certain pathways protect the nerve cell and release antioxidants to reduce oxidative stress. Nrf2 activation has a promising therapeutic effect on many diseases including neuroprotection by releasing more than 250 cytoprotective genes and also known as the master regulatory pathway of antioxidants. SIRTUIN 3 activation releases antioxidant genes SOD2, catalase to reduce ROS and increase mitochondrial biogenesis. AMPK activation promotes autophagy by ULK1 and activates PGC-1 $\alpha$  which is the master regulator of mitochondrial biogenesis. Heat shock protein activation promotes chaperon-mediated autophagy and macroautophagy to perform neuroprotection against accumulated protein.

**Keywords:** Neurodegeneration, Oxidative Stress, Nrf2, Heat Shock Protein

**Abbreviations:** NRF2: (Nuclear erythroid factor or Nuclear respiratory factor); KEAP-1 (Kelch-like ECH associating protein); BTB domain (Broad complex tram track & Bric a brack); IVR (The intervening region); DGR (Double glycine repeats); (bzip) basic leucine zipper CNC; maf (masculo-aponeurotic fibrosarcoma oncogene); ARE (Antioxidative response elements); (UBA) ubiquitin association; GSK-3 (Glycogen synthase Kinase 3); SOD2 (superoxide dismutase enzyme); OPA1 (Optic atrophy 1); PINK 1 (phosphatase and tensin homolog PTEN, induced kinase); NMNAT2 (Nicotinamide mononucleotide adenylyltransferase); NAD (Nicotinamide adenine dinucleotide); 3-TYP (Triazol pyridine); Trimethylamine-N-Oxide (TMAO), **5AMPK Activation** (Adenosine monophosphate-activated protein kinase), 00=(HMGCR) 3-Hydroxy 3-methylglutary Co-A reductase; TSC2 (Tumor suspension gene); mTOR (mammalian target of rapamycin); PGC-1 $\alpha$  (Peroxisome proliferator activator receptor gamma coactivator 1- alpha); CPT-1 (carnitine palmitoyltransferase-1); RabGAP (RabGTPase activating protein); ERR (Estrogen related receptor); (OMPs) outer membrane proteins HSP (Heat shock protein)

## Introduction

Parkinson's disease (PD) is a neurodegenerative condition characterised by the gradual loss of midbrain dopaminergic neurons, as well as mitochondrial failure, and is characterised by the cardinal symptoms of rigidity, bradykinesia, tremor, and postural instability [11]. Neurological diseases are currently the major cause of disability across the world. PD was the fastest increasing neurological illness in terms of prevalence, disability, and mortality in the Global Burden of Disease (GBD) research. According to recent studies, the global number of persons affected by the condition more than quadrupled between 1990 and 2016 [12].

### Nuclear factor erythroid 2-related factor 2 (Nrf2)

In both glial cells and neurons, NRF2 is an essential defender against injuries in the brain. As well as influencing several antioxidant enzymes, NRF2, Phase I and II drug metabolising enzymes and mitochondrial pathways can also enhance the expression of anti-inflammatory mediators [17].

Nrf2 knock-out mice, cortical culture has demonstrated they are susceptible to oxidative stress, Nrf2 re-expression increases neuroprotection through ARE activation [18].

### Nrf2 Expression in Neurodegenerative Diseases

According to a research done by Kaimin Chan et al., Nrf2 is expressed at low levels in many tissues, at high levels in the liver, lungs, and kidneys, and at the greatest levels in the cell lining of the digestive system, which extends from the oesophagus to the small and large intestines. In the CNS, Nrf2 is expressed in the lateral ventricle and its medial wall, as well as the choroid plexus in the fourth ventricle. Nrf2 expression is also found in the olfactory epithelium in the nasal cavity, the thyroid and submandibular glands, and the brown fat layer in the back. At higher magnification, NRF2 expression is most pronounced in the luminal cells of the stomach and intestine [19].

In Alzheimer's disease, Nrf2 is primarily expressed in cytoplasmic hippocampus neurons; it does not translocate to the nucleus; a biochemical extract of the frontal cortex confirmed decreased levels of nuclear Nrf2 in Alzheimer's disease. In the nuclei of Alzheimer's disease patients, neither neurons nor astrocytes showed substantial Nrf2 staining. The Nrf2 pathway is most likely dysfunctional in AD hippocampal neurons; nevertheless, in Parkinson's disease, Nrf2 is more numerous in dopaminergic

neurons than in SNpC (substantia nigra pars compacta), and dopaminergic Nrf2 exhibits neuronal sensitivity to increased oxidative stress [20].

### Nrf2 Structure

NRF2 (Nuclear factor erythroid 2-related factor 2) also called Nuclear factor erythroid-derived 2-like 2, was discovered in 1994 laboratory of Yuet Wai Kan, and it belongs cap "n" collar (CNC) subfamily of basic leucine zipper (bZIP) transcription factors and is encoded by NFE2L2 gene [1, 2]. It involves members possessing a homology region of CNC with 43 amino acids, which supports the N-term DNA-binding domain to the DNA-binding specificity of this family [3]. NRF2 is a modular protein that consists of 7 functional domains (Neh 1-7), as seen under *Figure 1*, known as the NRF2 ECH (Neh) domains, each of which has a distinct function [3, 4]. Neh1 contains a basic leucine zipper motif (bZIP) that heterodimerizes with small musculoaponeurotic fibrosarcoma protein, DNA, and other transcription partners, a study conducted by Plafker et al. Has shown that Neh1 of Nrf2 forms a nuclear complex with ubiquitin-conjugating enzyme UbcM2 utilizing DNA binding and regulating the stability of Nrf2 [5, 6]. Neh2 N-terminus interacts with the kelch domain of KEAP1 (Cullin (Cul)3-RING, dimeric redox-sensitive substrate adaptor Ubiquitin ligase complex including the box protein (Rbx)1 (i.e., CRLKeap1) through two binding sites, the stronger binding ETGE motif and the weaker binding DLG motif [7, 3, 10] shown in *Figure 2*. Studies show Interaction of C-terminal Neh3 with the transcription co-activator known as CHD6 (a chromo-ATPase/helicase DNA-binding protein) may play a key role in the activation of ARE driven gene [8]. Neh4 and Neh5 bind together to CBP (CREB-binding protein), synergistically stimulate gene expression via ARE and synchronizes reporter gene activation [9]. Neh6 contains two motifs, DSGIS and DSAPGS, which interact with dimeric bTrCP (b-transducin repeat-containing protein), which acts as a substrate receptor for S-phase kinase-associated protein 1 (Skp1)-Cul1-Rbx1 core E3 complex (i.e., SCFb-TrCP) shown in *Figure 3*, these two motifs are important for Nrf2 stability. It is important to note that Neh6 controls the stability of NRF2 in KEAP1 independent manner, Neh7 suppresses the NRF2-ARE signalling pathway by interacting with the retinoic X receptor alpha (RXR) [5, 10].

### Nrf2-KEAP1-ARE pathway

Oxidative stress is one of the major key player in chronic inflammation associated with neurodegeneration, The KEAP1-Nrf2 pathway is a major signaling pathway for antioxidant defense against reactive oxygen species assault. Nrf2 induces

expression of antioxidant enzyme genes such as glutathione S-transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), as well as hemeoxygenase-1 (HO1). This induction requires antioxidant response element (ARE) DNA sequence [13, 14], KEAP1 suppress activity of Nrf2 by binding to Neh2, it act as adaptor molecule for the Cul3 E3 ligase complex and leads to Nrf2 degradation through the ubiquitin–proteasome pathway, exposure to reactive oxygen species or electrophiles modifies KEAP1 and inactivates there by leading to attenuation of Nrf2 degradation [15], causing accumulation of Nrf2 in the nucleus and heterodimerization with small Maf proteins (MafF, MafG, and MafK) this substance binds to ARE at regulatory region of targeted gene [16,4] shown in *Figure 4*.

The regulation of Nrf2 is complicated, yet it can be regulated at several levels. Figure 1 depicts the transcriptional and post-transcriptional levels [4]. NRF2 abundance within the cell is tightly regulated and is mainly controlled by four E3 ubiquitin ligase complexes-mediated ubiquitylation and proteasomal degradation: KEAP1-Cullin (CUL) 3-RING-box protein (RBX)1,  $\beta$ TrCP-S-phase kinase-associated protein-1 (SKP1)-CUL1-RBX1, WD Repeat protein (WDR), 3-CUL4-damaged DNA binding protein (DDB) 1, and HRD1 (also called Synoviolin) under different conditions [23].

In unstressed condition its basal(homeostatic) protein level is low reason being KEAP1 mediated proteasomal degradation by Keap1/Cul3 E3 ubiquitin ligase complex [4,23], reactive oxidant species modifies specific cystin residue of KEAP1 (C257, C273, C288, and C297) causing disassociation of KEAP1 and Nrf2 migrate to nucleus causing its accumulation as evidenced in KEAP1 knockdown in human cells and KEAP1 deletion in

mice, and activation of cytoprotective genes [22,21,24,25].

Many cytoplasmic protein which have KEAP1 dependent but cysteine independent mechanisms have been identified, that stabilizes Nrf2 by attenuating interaction between KEAP1-Nrf2. P62 also known as Autophagy cargo-adaptor p62/sequestosome 1 (SQSTM1) [26,27,28,29,30,31]. dipeptidyl peptidase 3 (DPP3) [29], Wilms tumor gene on X chromosome (WTX) [32], and Partner and Localizer of BRCA2 (PALB2) [33] all contain KEAP1-interacting region (KIR)-like ETGE motifs and thus competes with NRF2 for KEAP1 binding, resulting in KEAP1 sequestration and NRF2 stabilization. P21 cyclin-dependent kinase p21Cip1/WAF1, interacts with DLG and ETGE inhibits KEAP1-Nrf2 binding [34]. BRCA1 promotes stability and activation of Nrf2 [35], P300 interacts with Nrf2 and interferes with KEAP1-Nrf2 complex formation [36].

NRF2 controls the expression of four genes, which include Glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, Malic enzyme 1, and Isocitrate dehydrogenase 1, which are responsible for the production of NADPH, which acts as a cofactor and fuel for antioxidant reactions. It also controls the expression of cytochrome P450 oxidoreductase. Among blood cells, monocytes and neutrophils have the greatest amounts of NRF2. In astrocytes, NRF2 levels are high. Furthermore, microglia, a monocyte lineage, exhibits higher amounts of NRF2 than neurons [49, 50, 51, 52].

NRF2 lowers inflammation through three different ways, redox metabolism modification, cross-talk with (NF-KB), direct regulation of pro-inflammatory genes [49, 53]. Table 1 gives list of Nrf2 activators.

Company/Compound	Comments	Ref
Reata /Bordoxolone Methyl	synthetic triterpenoid is in PhaseIII It shows pulmonary arterial hypertension .In phase II it shows chronic kidney failure and II diabetics	54
Reata/omaveloxolone	second-generation synthetic (2019) triterpenoid is in Phase for Friedreich's ataxia.	
Biogen: ALK8700	It is an prodrug of monomethylfumarte for multiple sclerosis is in Phase III	
Evgen Pharma: SFX-01	Cyclodextrin+Sulforaphane are in phase II for subarachnoid hemorrhage ; effective and safe In metastatic breast cancer with combination of tamoxifen and fulvestrant	
Anthocyanin	It is an flavonoid present In a garpes and berries used in atherosclerosis by binding keap for NRF <sub>2</sub> activation	
ITH12674	Hybrid molecule of melatonin+sulforaphane for brain ischemia by modify the Cys-151 of keap for NRF2 activation is in preclinicalstudies	

**Table 1:** List of NRF2 activators for different diseases

## SITRULIN-Nrf2

SIRT1 is the most extensively researched sirtuin, and it regulates several processes that govern eating and energy expenditure. When it is reduced, insulin resistance increases. If sirtuin1 levels rise, insulin sensitivity rises, sirtuin 1 deacetylates and promotes PGC1 alpha, ERR, which also has cell-protective action in both neuronal and non-neuronal cells by promoting autophagy and controlling cellular cholesterol, sirtuin3 levels rise, and NRF1, NRF2 levels rise. Which demonstrate a defence mechanism against ROS [55,56,57,58].

## Heat shock protein (HSP)

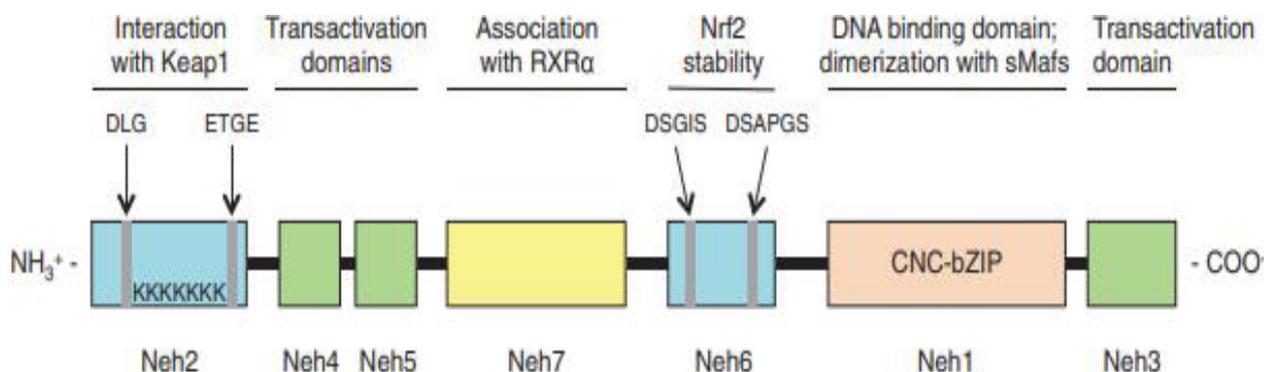
Heat shock proteins are a family of proteins produced by stressful conditions by cells. It is generally seen in heat accumulation / stress, cold stress, UV stress, during wound healing and tissue remodeling it activates molecular chaperones to regulate misfolding of new proteins and the negative effect on cellular protein caused by stressors such as high temperature, redox imbalance, heavy metals [59,60,61,62,63].

The upregulation of heat-shock protein is also said to be heat shock response and induced by heat shock factor. This HSP is found in all organisms from bacteria to humans. HSPs classified according to their molecular weight HSP60, HSP70, HSP90. Ubiquitin is a small protein for degradation also comes under HSP [64,65,66]. Activation of HSP in response to environmental stressors has been determined in bacteria. During heat stress, outer membrane proteins (OMPs) get misfolded and accumulated in periplasmic space which is detected by protease which passes the sigma signal transcription factor. Some studies show that accumulation or misfolding of proteins leads to activation of HSP [67]. RNA thermometer such as four thermometers, Rose

element, and Hsp90 regulatory element is the mechanism for upregulation of heat shock proteins in some bacteria.

Petersen and Mitchell experiment on *Drosophila melanogaster* to see the heat shock gene expression in heat stress as well as in cold stress and found that the HSP gene protects from death and enhances survival [68,69,70]. Molecular chaperones are the main function of heat shock protein. They regulate proper protein shape and correction of misfolding proteins by maintaining stabilize unfolded proteins. HSPs help to transfer proteins across the cell. All organism has some members of heat shock proteins family for protein maintenance [71,72]. HSP aids in monitoring proteins in the cell and they carry old proteins or damaged proteins to proteosomes for degradation and prevent new proteins from misfolding this activity are part of the cell repair system also known as a cellular stress response or heat shock response. Heat shock protein shows self-degradation on itself by proteolytic action [73]. HSP84, HSP90, HSP70, HSP27 has significant cardio protective role. HSP90 shows vascular relaxation by binding both soluble guanylate cyclase and endothelial nitric oxide synthase. Gata4 gene regulates cardiac genes *hspb12* and *hspb7* which are responsible to prevent cardiac myopathies in embryos of zebrafish are observed and show cardiac morphogenesis. HSPb7 also known as cardiac heat shock protein and act on kupffer vesicles for regulation of left-right asymmetry of zebrafish heart. Hsp20 shows a major role in the prevention of platelet aggregation, cardiac myocytes function, skeletal muscle function, muscle insulin response, and prevention of apoptosis after ischemia injury [74,75,76].

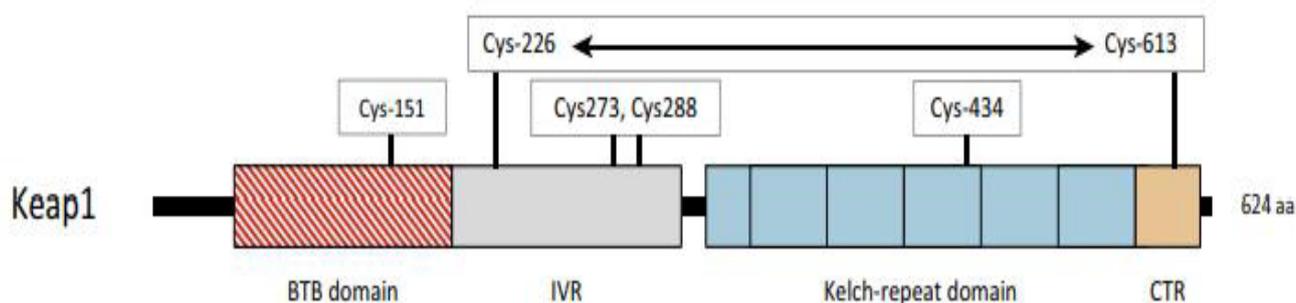
Macroautophagy and chaperon-mediated autophagy is promoted by HSPs when damaged protein aggregate and misfolding of synthesized protein [77,78]. Table 2 referenced different pathways for neuroprotective activity.



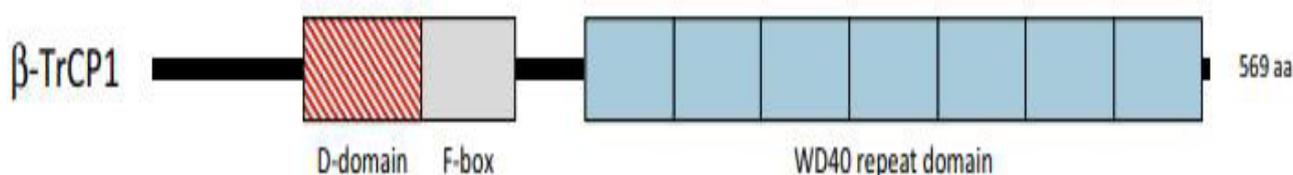
**Figure 1:** human Nrf2 protein structure. The Nrf2 protein is made up of seven Neh domains. The Neh1 CNC-bZIP domain is in charge of DNA binding and dimerization with small Maf proteins; the Neh2 domain mediates interaction with Keap1 via the DLG and ETGE motifs and contains seven lysine residues that are ubiquitylated; the Neh3, Neh4, and Neh5 domains are transactivation domains; the Neh6 domain is a serine-rich region that regulates Nrf2 stability; and the Neh7 domain bZIP, basic-region leucine zipper; CNC, cap "n" collar; Keap1, Kelch-like ECH-associated protein 1; Neh, Nrf2-ECH homology; Nrf2, nuclear factor E2-related factor 2; RXRa [4].

TITLE	REFERENCE
Nrf2-ARE pathway: An emerging target against oxidative stress and neuroinflammation in neurodegenerative diseases. Pharmacol. Ther.	37
Nrf2-Keap1 signaling in oxidative and reductive stress	38
The Nrf2/ARE pathway: A promising target to counteract mitochondrial dysfunction in Parkinson's disease. Parkinson's	39
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Orientin-mediated Nrf2/HO-1 signal alleviates H <sub>2</sub> O <sub>2</sub> -induced oxidative damage via induction of JNK and PI3K/AKT activation.	48

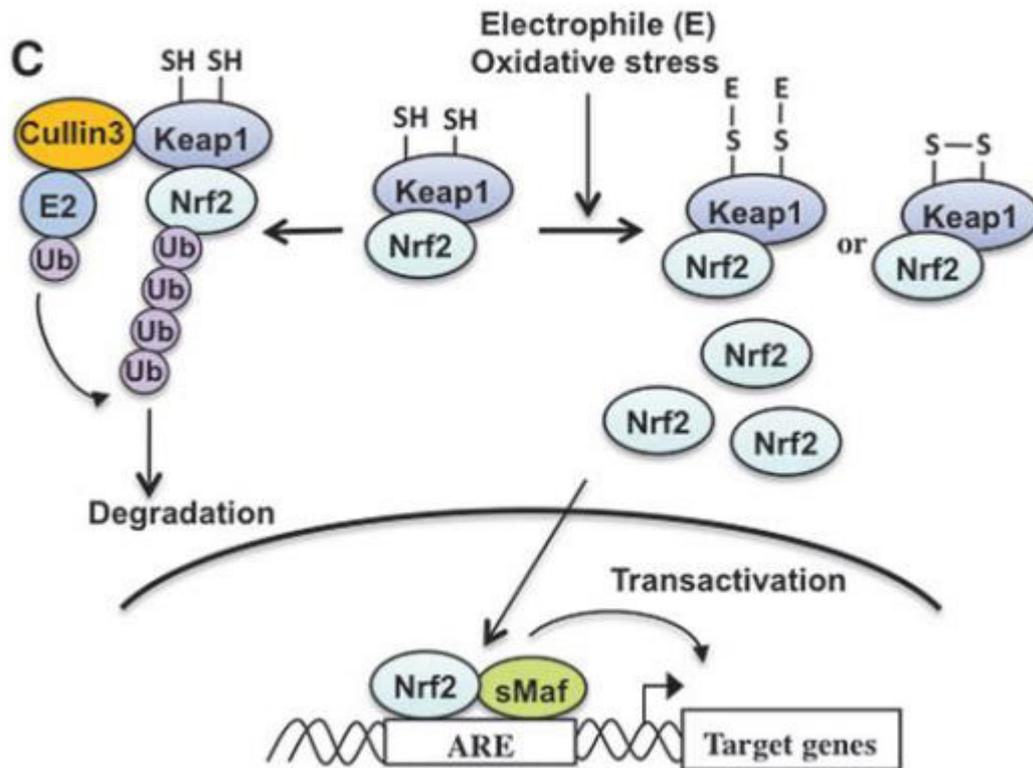
**Table 2:** Different pathways upon triggering shows neuroprotective activity



**Figure 2:** The domains of Keap1 are displayed in their current locations. The Bric-a'-Brac (BTB) domain (shown as a striped red box) is responsible for the formation of Keap1 homodimers and the recruitment of Cullin-3, whereas the Kelch-repeat domain (each Kelch domain is depicted as a blue box) and the C-terminal region (CTR, depicted in light orange) form a six-bladed  $\beta$ -propeller structure that binds the DLG and ETGE motifs in Nrf2. The intervening region (IVR), which wraps around the  $\beta$ -propeller structure, is made up of residues between the BTB and Kelch-repeat domains. Residues that function as sensors for electrophiles and H<sub>2</sub>O<sub>2</sub> (i.e., Cys-151, Cys-226/Cys-613, Cys-273/Cys-288, and Cys-434) are shown in open boxes above the cartoon, and the horizontal two-headed arrow between Cys-226 and Cys-613 signifies that they form a disulfide bridge when exposed to H<sub>2</sub>O<sub>2</sub> [10].



**Figure 3:** The domain locations in b-TrCP1 are shown. A striped red box represents the dimerization domain (D domain), which produces homo- and heterodimers between b-TrCP1 and b-TrCP2, and a grey box represents the F-box, which recruits Skp1 (S-Phase Kinase-Associated Protein 1). Finally, the blue boxes indicate the WD40 repeat domain, which binds the DSGIS and DSAPGS motifs in Nrf2 [10].



**Figure 4:** The mechanism of Nrf2 activation is depicted in this diagram. Nrf2 is constitutively ubiquitinated by the Keap1=Cullin3 ubiquitin ligase complex in non-stressed circumstances. Keap1 is inactivated and changed at its regulatory cysteine residues when exposed to electrophile (E) or oxidative stress. Inactivation of Keap1 promotes nuclear accumulation of Nrf2 and ARE-dependent transcription. [15].

## Conclusion

Neurological disorders such as Parkinson's and Alzheimer's are primarily caused by oxidative stress or redox imbalance, in which free radicals or ROS react with cellular lipids, proteins, and DNA, resulting in cell death. Protein accumulation, mitochondrial dysfunction, proteasomal dysfunction to mitigate these parameters, all of these factors contribute to cell death and neurological disorders. Different detoxification mechanisms can minimise oxidative stress and sustain mitochondrial biogenesis while boosting autophagy for the destruction of accumulated proteins and misfolded proteins.

## Conflicts of interest

The authors declare no conflicts of interest concerning authorship or publication of this article

## Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as

honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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