

RESEARCH

Open Access



Neuroprotective effects of green tea (*Camellia sinensis*) and broccoli (*Brassica oleracea*) extracts against Cuprizone-induced cytotoxicity in neuroblastoma (SH-SY5Y) cells

Sara Fathallah¹ and Ahmed Abdellatif^{1,2*} 

Abstract

Background Natural antioxidants are crucial in slowing the progression of nervous system disorders such as multiple sclerosis (MS), primarily by counteracting oxidative stress and neuroinflammation—two central drivers of neuronal injury. Green tea (EGCG) and broccoli (sulforaphane, SF) extracts are rich in potent antioxidant and anti-inflammatory compounds, yet their comparative neuroprotective efficacy against cuprizone (CPZ)-induced toxicity has not been thoroughly investigated.

Methods GC–MS confirmed catechins and caffeine as the main green tea constituents, and sulforaphane with flavonoids as predominant in broccoli. SH-SY5Y neuroblastoma cells were exposed to CPZ (750 or 1000 μ M) with or without extract pretreatment (EGCG, 5 or 10 μ M; SF, 10 or 20 μ M). Cell viability (MTT, trypan blue), membrane/nuclear morphology (light and fluorescence microscopy), and expression of MMP-3, IL-1 β , NF- κ B1, HIF-1 α , and miR-124-3p were assessed.

Results CPZ (750 μ M) reduced viability by > 80% and induced marked structural damage. Optimal doses—EGCG 5 μ M and SF 10 μ M—significantly preserved cell viability and morphology. CPZ elevated MMP-3 and suppressed NF- κ B1, HIF-1 α , and miR-124-3p, with no change in IL-1 β . EGCG alone markedly reduced MMP-3 and IL-1 β ; SF alone lowered IL-1 β and moderated miR-124-3p loss. SF + CPZ broadly attenuated CPZ effects, significantly reducing MMP-3 and IL-1 β and restoring NF- κ B1 and HIF-1 α . EGCG + CPZ conferred selective protection but increased MMP-3 relative to EGCG alone.

Conclusion Both extracts mitigate CPZ-induced neurotoxic signaling via distinct yet overlapping pathways—EGCG through targeted suppression of matrix degradation, SF through broader anti-inflammatory and gene-restorative effects. The current findings support their potential as complementary neuroprotective strategies, warranting further in vivo validation.

Keywords Neurotoxicity, Green tea, *Camellia sinensis*, Broccoli, *Brassica oleracea*, Neuroprotection

*Correspondence:
Ahmed Abdellatif
Ahmed.abdellatif@aucegypt.edu

¹Biotechnology Program, The American University in Cairo, Cairo 11835, Egypt

²Department of Biology, School of Sciences and Engineering, The American University in Cairo, Cairo 11835, Egypt

Introduction

Multiple sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS), characterized by the infiltration of T-cells, B-cells, and monocytes into the CNS as well as oligodendrocyte damage [1], leading to demyelination and CNS functional changes including, cognitive impairment, anxiety, depression, and other neurological symptoms [2, 3]. Current MS treatment is aimed at alleviating the disease's symptoms and delaying its progression. Available therapies have significant side effects [4, 5]. For example, Aubagio and Ocrevus inhibit de novo pyrimidine synthesis, therefore affecting T and B cell function and negatively impacting the immune system, in addition to causing liver failure, seizures, and depression [6, 7].

Natural antioxidants play a pivotal role in mitigating the progression of nervous system disorders, particularly multiple sclerosis (MS), by counteracting oxidative stress and neuroinflammation, two key drivers of neuronal damage. Compounds such as curcumin (from turmeric), green tea polyphenols, and vitamins C and E have demonstrated neuroprotective effects by scavenging free radicals and modulating inflammatory pathways. Curcumin, for instance, inhibits pro-inflammatory cytokines like TNF- α and IL-1 β [8], while enhancing antioxidant enzymes such as superoxide dismutase and glutathione peroxidase, thereby preserving myelin integrity and reducing neurodegeneration in MS. Additionally, vitamin D and B12 supplementation have shown promise in supporting immune regulation and myelin repair, both critical in MS management. These natural agents, when integrated thoughtfully into treatment plans, may complement conventional therapies and improve the quality of life for individuals with MS [9].

Green tea (*Camellia sinensis*) and Broccoli (*Brassica oleracea*) are potent antioxidants due to the presence of Epigallocatechin gallate (EGCG) in the former and sulforaphane (SF) in the latter. Both SF and EGCG exert their antioxidant activity by activating endogenous antioxidative enzymes such as Glutathione (GSH) and Nuclear response factor 2 (Nrf2) pathway, and by inducing resistance to mitochondrial permeability. They also reduce the expression of inducible nitric oxide synthase (iNOS) [10]. SF is neuroprotective in experimental models of MS [11, 12]. Therefore, these naturally occurring substances are of great potential as neuroprotective agents and can be used to improve the quality of life in MS patients.

In this study, we used the human neuroblastoma SH-SY5Y cell line, a widely used in vitro model extensively utilized to investigate neuroinflammation, neurodegeneration, neurotoxicity, oxidative stress, and neuroprotection [13]. These cells have also served as a platform for examining the effects of cuprizone (CPZ)

on neural cells in vitro [14] and as a model for neurotoxic and neurodegenerative disorders, including Parkinson's and Alzheimer's diseases [15] de [16]. Importantly, SH-SY5Y cells offer human-specific relevance and reproducibility that are difficult to achieve with primary rodent neurons, making them a robust and efficient system for exploring cellular responses to CPZ and plant-derived neuroprotective agents [17–19].

Cuprizone (CPZ) is a copper chelator that has been used to induce demyelination and to create both in vitro and in vivo models of MS [20]. CPZ exerts its toxicity by causing mitochondrial damage [21] and by triggering inflammation and inducing inflammatory cytokines that cause the damage [22]. Previous reports [23] analyzed the effect of CPZ in neuronal cellular models, showing that CPZ did not cause loss of cell viability in neuroblastoma (SH-SY5Y) and Mouse GnRH Neuronal Cell Line (GN11) [23]. Later studies showed that the proliferative and survival rates of SH-SY5Y, microglia, astrocytes, and oligodendrocyte precursor cells were not affected by short-term treatment with different concentrations of CPZ [24]. Recently, CPZ was also found to induce a dose-dependent reduction in cell viability, mitochondrial function, and aerobic cell respiration, thus compromising cell metabolism in neural cells and neuron-glia co-cultures [20]; [25]. These discrepancies in cytotoxicity may be due to variations in methodology, concentration, or exposure time to CPZ.

The nervous system normally has low or undetermined levels of MMP-3, IL-1 β , and MicroRNA-124-3p [26] Ristori and Stefania [27]. IL-1 β contributes to the inflammatory response in the nervous tissue and is correlated with both the number and volume of demyelinating lesions in the brain cortex (Lin and Edelson [27–29]). Some of the drugs used to treat MS affect IL-1 β levels, such as Glatiramer acetate (GA), type 1 IFN, and Natalizumab (anti-VLA-4). Furthermore, MMP-3 expression was also increased in the MS animal model induced by CPZ [30]. MiR-124 is also greatly downregulated in neurotoxicity [31]. While its overexpression enhanced cell viability and inhibited neuronal apoptosis, and improved the neurological function in rats [32].

Nuclear factor KB (NF- κ B1) has a major role in the control of physiological processes in the CNS and is associated with the development of spatial memory, synaptic transmission, and neuroplasticity. Many CNS diseases, such as epilepsy, cerebral ischemia, Alzheimer's disease, Parkinson's disease, and Huntington's disease, have all been linked to NF- κ B1 [33, 34].

HIF-1 α expression is also associated with the pathophysiology of inflammatory demyelination because it controls the transcription of a large number of genes involved in neuroprotection and neuroinflammation [35, 36]. HIF-1 α gene expression has also been reported to

be dramatically downregulated in the Cuprizone-treated neurons. Suppression of HIF-1 α also reduced the Experimental autoimmune encephalomyelitis (EAE) symptoms because it affected the oligodendrocytes and T-cells. So, the action of HIF-1 α depends on the cell type, but further studies are needed to fully understand the role of HIF-1 α in MS [37].

In the current study, we examined the expression of MMP-3, IL-1 β , HIF-1 α , and NF- κ B1 genes as well as miRNA 124-3p in a Cuprizone-induced neurotoxicity model using a single cell culture of neuroblastoma (SH-SY5Y) cells, since these markers (MMP-3, IL-1 β , HIF-1 α , and NF- κ B1 genes as well as miRNA 124-3p) may be valuable in MS diagnosis and prognosis. The neuroprotective effects of Broccoli and Green tea extracts were also assessed on neuroblastoma cells since Broccoli and green tea plant extracts have been reported to reduce the expression of MMP-1, MMP-2, MMP-3, NF- κ B1, and IL-1 β .

Materials and methods

Plant extraction and characterization

Broccoli extract (*Brassica oleracea*) was prepared from natural supplement capsules (Best Naturals, NJ, USA). Each 1000mg capsule contained 0.3% sulforaphane (molecular weight 177.29g/mol), equivalent to 3mg [38].

Green tea extract (*Camellia sinensis*) was similarly obtained from natural supplement capsules (SilverOnyx, UT, USA). Each 1000mg capsule contained 98% EGCG (molecular weight 458.375g/mol), corresponding to 980mg [39].

For experimental use, 1mM stock solutions were prepared by dissolving 17.7mg of broccoli extract and 46.77mg of green tea extract separately in 100mL of phosphate-buffered saline (PBS) [38, 39]. EGCG dissolved readily in PBS, whereas the broccoli extract contained insoluble impurities and was centrifuged at 3000 rpm for 10min. The resulting supernatant from each extract was sterilized by filtration through a 0.2 μ m syringe filter. Prepared stock solutions were aliquoted and stored at -20 °C until use.

For further analysis, one gram of the dry powder (Green tea and Broccoli) was weighed and mixed with 50mL of ethanol for 9 hrs, and the solution was gently refluxed and then allowed to cool. The mixture was filtered and evaporated to dryness in a rotary evaporator system. The extract yield was dissolved again in ethanol and transferred to a GC-MS vial [40]. Both solutions were analyzed using a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30m $\times$ 0.25mm $\times$ 0.25 μ m film thickness) to identify the structure and abundance of the active ingredients.

In vitro studies

Human neuroblastoma SH-SY5Y cells (ATCC CRL-2266) were grown in RPMI media with 2mM L-glutamine, 5% 100 units/mL streptomycin/penicillin, 1% nonessential amino acids, and 10% (v/v) heat-inactivated FBS, which can be called complete RPMI media. Cells were maintained at 37 °C with 5% CO₂. Cells were treated with plant extracts (SF 5 -100 μ M, and EGCG 2.5 - 100 μ M) and CPZ 500 - 1500 μ M (CPZ, Sigma Aldrich, USA, molecular weight is 278.35g) [21]. Control cells were treated with complete RPMI media only.

Cell viability was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. The absorbance was measured at 570 nm using a SPECTROstar® Nano plate reader. Cell viability was plotted as the percentage of the controls [14].

For cell counting, cells were collected and resuspended in complete RPMI media. Twenty microliters of the cell suspension was mixed with 20 microliters of trypan blue (0.4%) and counted using a hemocytometer under a light microscope.

DAPI (4',6-diamidino-2-phenylindole) was used to assess the morphological changes in SH-SY5Y cells. Neuroblastoma cells were treated with green tea, broccoli, and CPZ at 5 μ M, 10 μ M, and 750 μ M, respectively. Cells were collected after 24 hours and fixed with 100 μ L of methanol for 10 minutes. Cells were washed with 100 μ L of PBS and 20 μ L of 300nM DAPI solution and incubated for 20 minutes, followed by washing with PBS. Cells were examined under a fluorescent microscope (Leica Microsystems, Germany).

Quantitative reverse transcription PCR (qRT-PCR)

Cells treated with plant extracts (SF 5 -100 μ M, and EGCG 2.5 - 100 μ M) and Cuprizone (500 - 1500 μ M) were harvested, and RNA was extracted using the RNeasy mini kit (Qiagen, catalog number: 74104). RNA concentration (OD) was measured using a SPECTROstar® Nano plate reader. The 260/280 ratio ranged from 1.9–2.0, confirming the purity of RNA. miRNA was extracted from the RNA using the miRNeasy mini kit (Qiagen, catalog number 217004).

An Omniscript kit (Qiagen, catalog number: 205111) was used for reverse transcription of the mRNA. The condition was set for 60 minutes at 37.0 °C, and storage at 4.0 °C forever. The product was stored immediately at -20.0 °C. miRCURY LNA RT Kit (catalog number: 339340) was used for the reverse transcription of miRNA. cDNA was stored at -20.0 °C until use.

Quantitative reverse transcription PCR (qRT-PCR) was conducted to measure the expression levels of 4 genes, MMP-3, IL-1 β , HIF-1 α , and NF- κ B1, and miRNA 124-3p in the six different conditions: Control cells, CPZ-treated

cells, EGCG-treated cells, SF-treated cells, and the EGCG/CPZ and SF/CPZ combinations.

Gene-specific primers (supplementary material, Tables [s1](#) & [s2](#)) were designed using the NCBI BLAST primer designing tool. The miRNA primers were extracted from QIAGEN GeneGlobe. Hera Plus SYBR Green (Willowfort, UK, catalog number: WF10308001) was used for qRT-PCR (Applied Biosystem 7500). GAPDH was used as a housekeeping gene to normalize the CT values and obtain the fold change in the gene expression according to the $2^{-\Delta\Delta C_t}$ method [41]. For miRNA, the miRCURY LNA SYBR Green PCR Kit (Qiagen, catalog number: 339345) was used. Endogenous control SNORD38B was used for the normalization of CT values and to obtain the fold change in the gene expression according to the $2^{-\Delta\Delta C_t}$ method [41].

Statistical analysis

GraphPad Prism version 9 (San Diego, CA, USA) was used to analyze the data. A one-way ANOVA test was used for multiple comparisons. Data are represented as mean $\pm$ standard deviation. P-values < 0.05 were considered significant. One-way ANOVA test was used to analyze the results of the trypan blue assay, MTT assay, and the values of the qRT-PCR.

Results

Plant extraction and characterization

GC–MS analysis

identified catechins—epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC)—along with caffeine as the major constituents of the green tea extract. These compounds possess well-documented neuroprotective, anti-inflammatory, anticancer, antibacterial, and immunomodulatory properties.

The broccoli extract was rich in sulforaphane (SF), a potent activator of the Nrf2 pathway that stimulates the expression of endogenous antioxidant enzymes, thereby counteracting oxidative stress and inflammation. In addition to SF, the extract contained rutin and glucosyl luteolin, both recognized for their strong antioxidant and anti-inflammatory activities.

The key constituents of the green tea and broccoli extracts are summarized in Tables [1](#) and [2](#), respectively.

Determination of IC_{50} and extract safety

The half-maximal inhibitory concentration (IC_{50}) of cuprizone (CPZ) and the safety profile of broccoli sprout extract (SF) and green tea extract (EGCG) were evaluated using the MTT cell viability assay (Supplementary Fig. [1](#)). SH-SY5Y neuroblastoma cells were treated for 24 h with SF (10 or 20 μ M) or EGCG (5 or 10 μ M), while CPZ was applied at 750 or 1000 μ M.

At the tested concentrations, SF and EGCG did not significantly affect cell viability compared with untreated controls. In contrast, CPZ at 750 μ M caused a marked reduction in viability ($p < 0.0001$; Supplementary Figure [S1](#)).

Cytotoxicity and trypan blue exclusion assays confirmed that CPZ (750 μ M) induced approximately 80% cell death ($p < 0.0001$). Treatment with either SF or EGCG significantly increased cell viability above control levels ($p < 0.0001$), and pre-treatment with either extract effectively reversed CPZ-induced damage. EGCG conferred greater protection than SF ($p < 0.0001$ and $p = 0.0064$, respectively, Fig. [1](#)).

Protective effects of extract pretreatment

Pretreatment with EGCG (5 μ M) or SF (10 μ M) before CPZ exposure (750 μ M) significantly increased cell viability compared to CPZ alone ($p < 0.0001$, Fig. [1](#)). In contrast, SF (20 μ M) and EGCG (10 μ M) did not confer significant protection. EGCG 5 μ M and SF 10 μ M were identified as the most effective concentrations for preventing CPZ-induced cytotoxicity and were selected for further experiments.

Microscopy and trypan blue assay findings

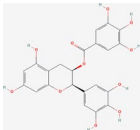
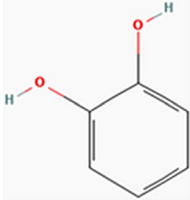
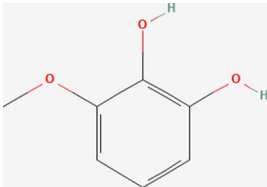
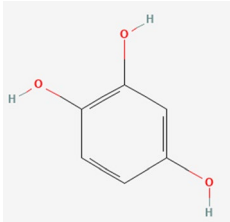
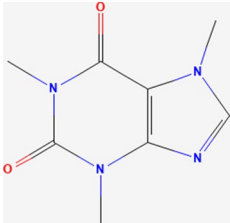
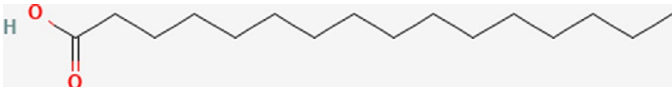
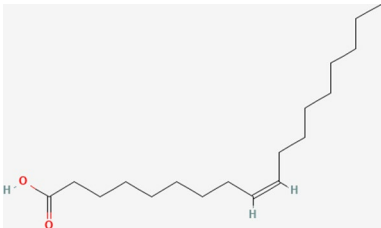
Microscopic and viability assay assessment of SH-SY5Y neuroblastoma cells following cuprizone (CPZ) and extract treatments. Light microscopy revealed that CPZ (750 μ M) induced extensive cell death, which was prevented by pretreatment with broccoli extract (SF, 10 μ M) or green tea extract (EGCG, 5 μ M). Trypan blue exclusion confirmed $> 80\%$ cell death with CPZ ($p < 0.0001$). Both extracts significantly improved cell viability ($p < 0.0001$), with EGCG providing greater protection than SF ($p < 0.0001$ and $p = 0.0064$, respectively).

Dapi staining observations

Fluorescence microscopy of DAPI-stained cells showed that Control cells exhibited intact membranes and nuclei with preserved cell–cell contacts (Figs. [2](#) CPZ treatment: loss of membrane integrity and nuclear structure (Fig. [2](#)). In the Broccoli and green tea treatment, preserved membrane integrity and promoted cell growth, with green tea demonstrating greater protection. Pretreatment with SF 10 μ M or EGCG 5 μ M prevented CPZ-induced damage, with EGCG offering stronger protection (Fig. [2](#)).

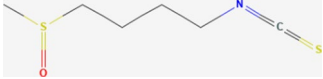
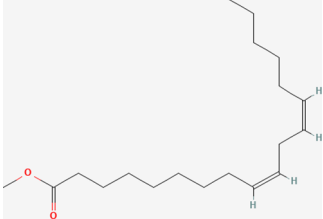
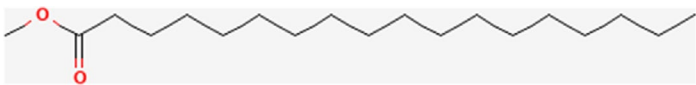
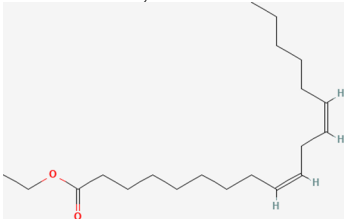
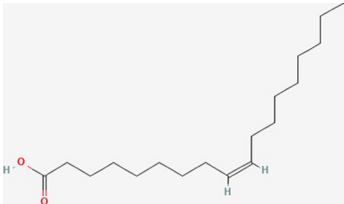
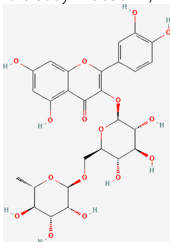
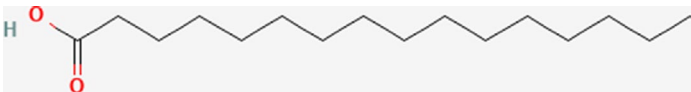
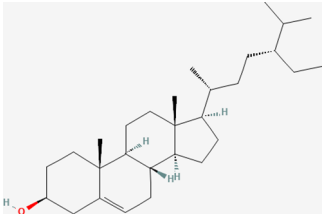
The MTT assay, microscopy, and trypan blue exclusion collectively demonstrate that EGCG (5 μ M) and SF (10 μ M) effectively protect SH-SY5Y neuroblastoma cells from CPZ-induced cytotoxicity. Both extracts preserved membrane integrity, nuclear morphology, and cell–cell contacts, with EGCG exhibiting a greater protective effect than SF. These concentrations were optimal for

Table 1 Active ingredients in the green tea extract

RT (Retention time min)	Mol. weight	Compound	Biological use	Pubchem ID
4.58		Epigallo-catechin gallate EGCG* (C ₂₂ H ₁₈ O ₁₁)	Antioxidant, Anti-inflammatory [42]	65064
				
9.74	110	Benzene- diol Catechol (C ₆ H ₆ O ₂)	Antioxidant [43]	289
				
10.99	140	Pyrocatechol (C ₇ H ₈ O ₃)	Antioxidant [44]	13622
				
14.24	126	Benzenetriol (C ₆ H ₆ O ₃)	Antioxidant	10787
				
24.38	194	Caffeine (C ₈ H ₁₀ N ₄ O ₂)	Antioxidant & neuro-protective [45]	2519
				
26.99	256	Palmitic acid (C ₁₆ H ₃₂ O ₂)	Antioxidant and neuroprotective	985
				
30.29	282	Oleic acid (C ₁₈ H ₃₄ O ₂)	Antioxidant	445639
				

*Epigallocatechin gallate EGCG is the combination of benzene diol-catechol, benzentriol, and pyrocatechol

Table 2 Active ingredients in the broccoli extract

RT (retention time min)	Mol. weight g	Formula	Biological use	Pub-chem ID
18.71	177	Sulforaphane (C ₆ H ₁₁ NOS ₂) 	Antioxidant [46]	5350
29.32	294	Sterculic acid, methyl linoleate C ₁₉ H ₃₄ O ₂ 	Antioxidant and antifungal [47]	5284421
29.98	298	methyl stearate C ₁₉ H ₃₈ O ₂ 	Antioxidant [48]	8201
30.53	308	Linoleic acid ethyl ester C ₂₀ H ₃₆ O ₂ 	Antioxidant [47]	5282184
38.63	282	Oleic acid C ₁₈ H ₃₄ O ₂ 	Antioxidant [49]	445639
41.55	610	Glucosyl Luteolin, Rutin (C ₂₇ H ₃₀ O ₁₆) 	Anti-tumor, anti-oxidant, Anti-inflammatory, and neuroprotective [50, 51]	5280805
42.54	496	Palmitic acid (C ₃₂ H ₆₄ O ₃) 	Antioxidant and neuroprotective [52]	71384704
45.75	414	Sitosterol (C ₂₉ H ₅₀ O) 	Antioxidant [53]	222284

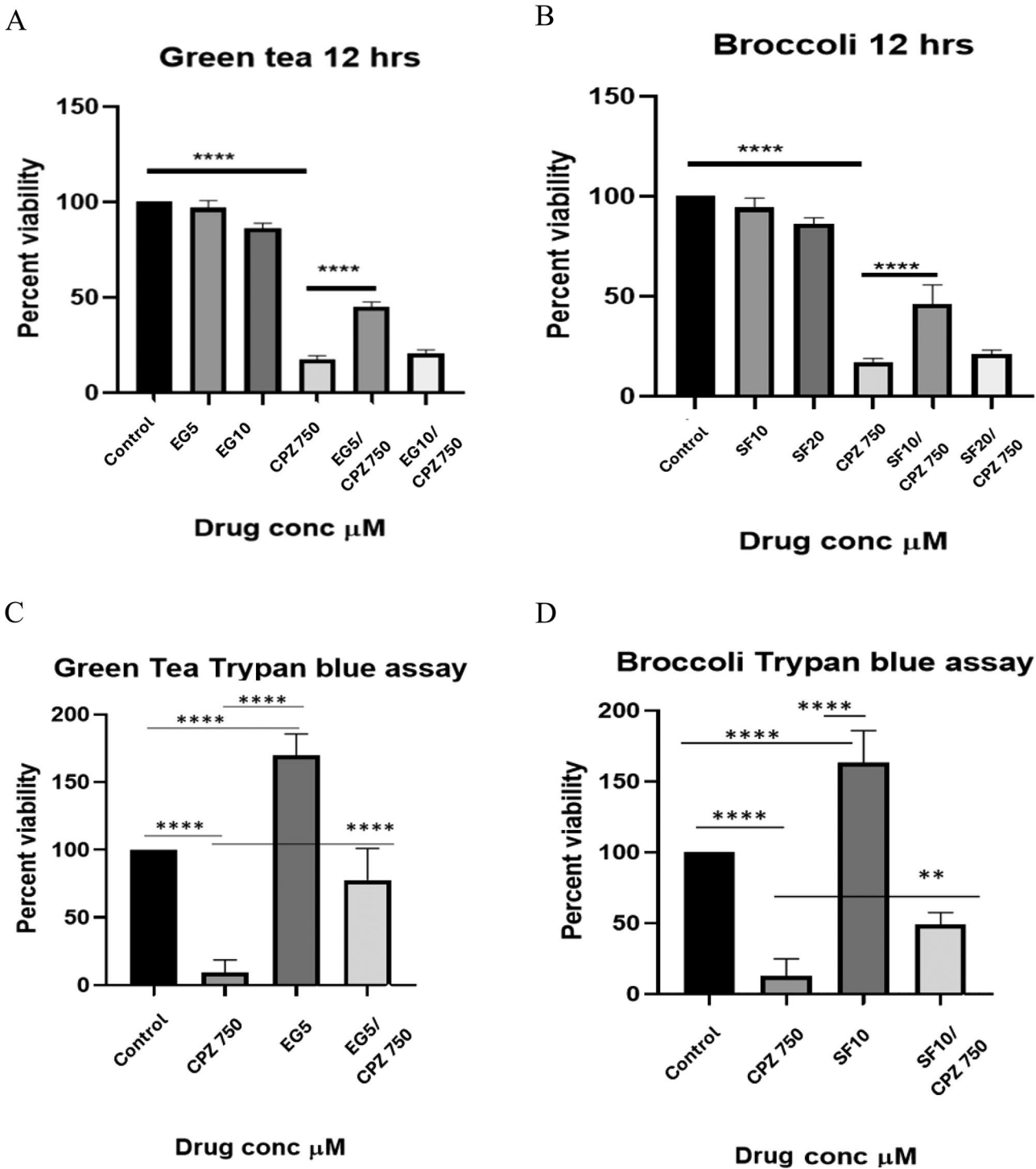


Fig. 1 Assessment of the neuroprotective effects of the plant extracts. Cytotoxicity and trypan blue exclusion assays assessing the protective effects of broccoli extract (SF) and green tea extract (EG) against cuprizone (CPZ)-induced cell death in SH-SY5Y neuroblastoma cells. Exposure to cuprizone (CPZ, 750 μM) for 24 h resulted in ~80% cell death ($p < 0.0001$). Treatment with sf (10 μM) or eg (5 μM) significantly increased cell viability above control levels ($p < 0.0001$). Pre-treatment with either extract markedly reversed CPZ-induced cytotoxicity, with eg providing greater protection than sf ($p < 0.0001$ and $p = 0.0064$, respectively). Data are presented as mean $\pm$ SD; $n = 4$

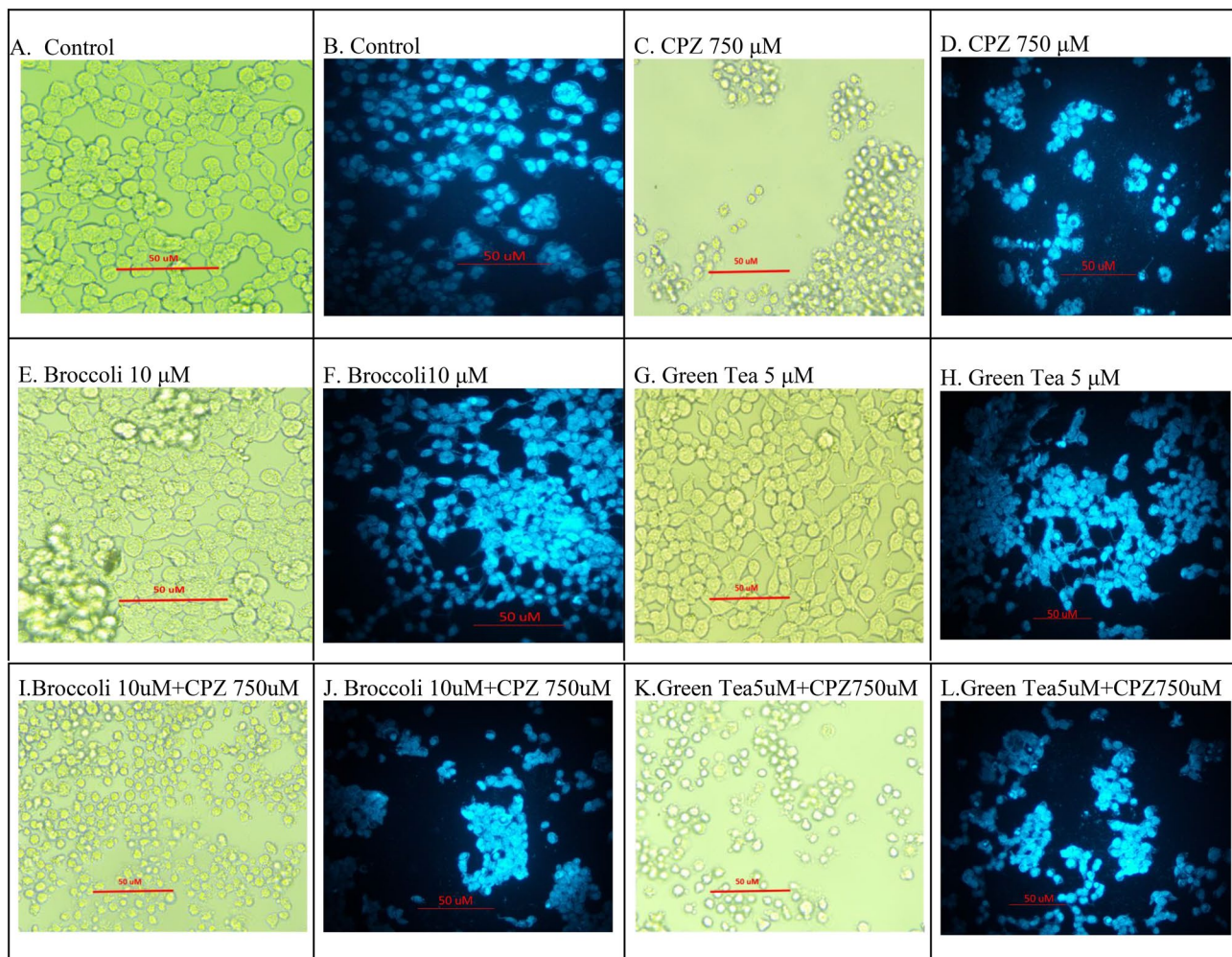


Fig. 2 Microscopic examination of neuroblastoma cells (SH-SY5Y). microscopic analysis of SH-SY5Y neuroblastoma cells following 48 h treatment. Control cells displayed normal morphology with intact membranes and nuclei (A, B). Exposure to cuprizone (CPZ, 750 μ M) induced pronounced cell death and structural alterations in the plasma membrane and nucleus (C, D). Treatment with broccoli extract (10 μ M; E, F) or green tea extract (5 μ M; G, H) promoted cell growth and preserved morphology relative to control and CPZ-treated cells. Combined treatments of broccoli 10 μ M+CPZ 750 μ M (I, J) or green tea 5 μ M+CPZ 750 μ M (K, L) mitigated CPZ-induced cytotoxicity, with the broccoli + CPZ combination showing greater cell survival than the green tea + CPZ combination. Images captured at 400 $\times$ magnification; $n=4$

mitigating CPZ-driven cell death and will serve as the basis for subsequent mechanistic investigations.

Gene and microRNA expression analysis

MMP-3 – Matrix metalloproteinase-3 levels were significantly elevated in CPZ-treated cells compared to control (Fig. 3, $p=0.0006$). Green tea extract (EGCG) alone significantly reduced MMP-3 expression relative to both control ($p=0.01$) and broccoli extract (SF) ($p=0.0011$), while SF alone showed no significant difference from control. Combination treatments—EGz (EGCG + CPZ) and SFz (SF + CPZ)—significantly downregulated MMP-3 compared with CPZ alone ($p=0.0226$ and $p<0.0001$, respectively). SFz also significantly decreased MMP-3 expression compared to SF alone ($p=0.0002$), whereas

EGz significantly increased MMP-3 compared to EGCG alone ($p=0.0004$) (Fig. 3).

IL-1 β – No significant difference was observed between CPZ-treated and control cells. However, SF or EGCG treatment alone significantly reduced IL-1 β expression compared to control ($p=0.0021$ and $p=0.0003$, respectively). In combination treatments, SFz significantly decreased IL-1 β levels compared to CPZ ($p=0.0337$) and control ($p=0.005$) (Fig. 3), while no significant change was seen between CPZ and EGz.

NF- κ B1 – Expression was significantly downregulated in CPZ-treated and EGCG-treated cells compared with control ($p=0.0014$ and $p=0.002$, respectively). In contrast, SFz significantly upregulated NF- κ B1 compared to CPZ alone ($p=0.0086$) (Fig. 3). No significant changes

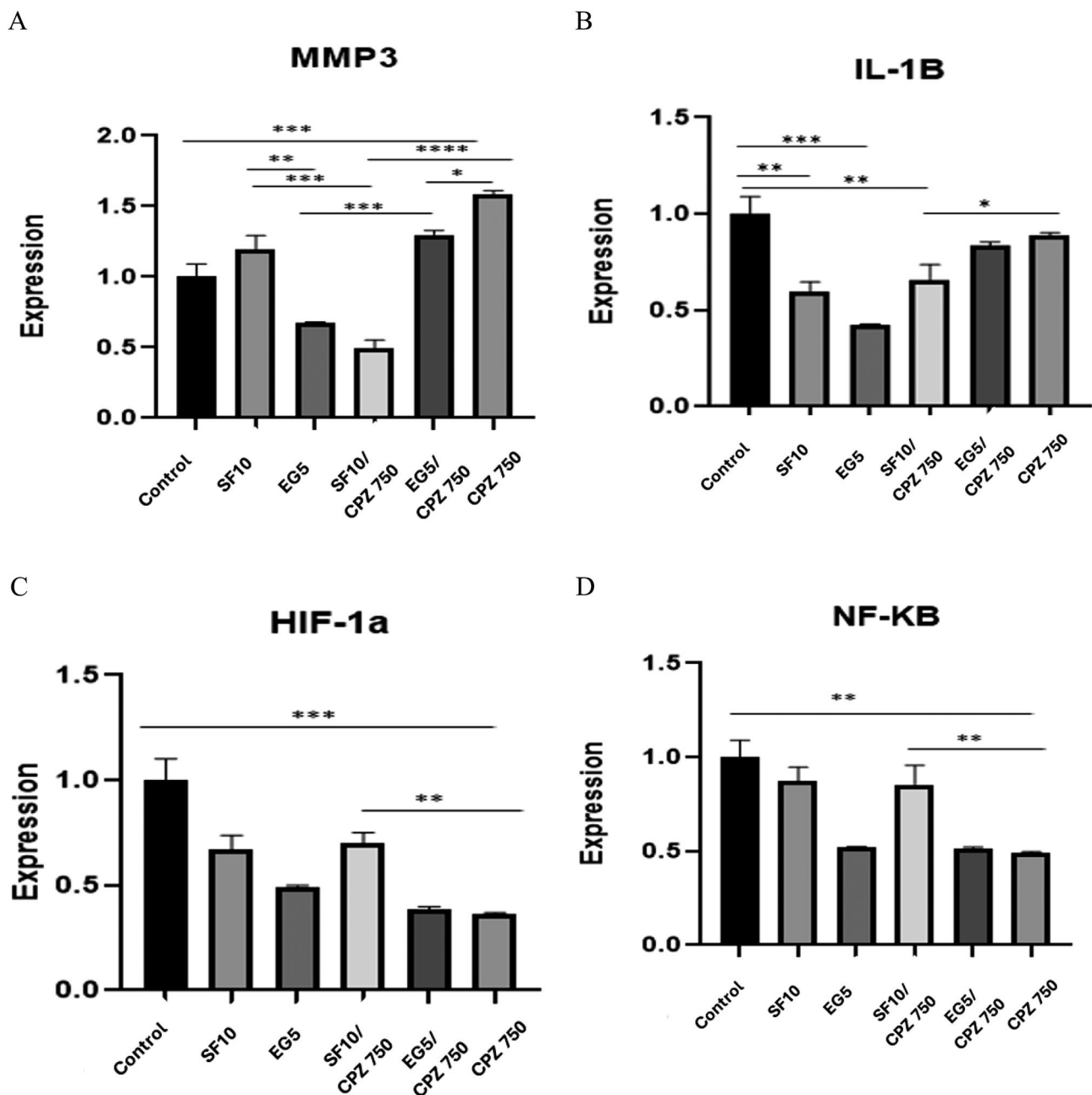


Fig. 3 Quantitative pcr (qRT-PCR) of MMP-3, IL1-B, HIF-1α, and NF-κB mRNA. Quantitative RT-PCR analysis of mmp-3, IL-1β, HIF-1α, and NF-κB1 mRNA expression in SH-SY5Y neuroblastoma cells following cuprizone (CPZ) and extract treatments. CPZ (750 μM) significantly upregulated mmp-3 ($p=0.0006$) without altering IL-1β expression. The green tea extract (eg, 5 μM) markedly reduced mmp-3 levels compared with control and broccoli extract (sf, 10 μM) ($p=0.0011$), while both SF and EG significantly decreased IL-1β relative to control ($p=0.0021$ and $p=0.0003$, respectively). The SF10/CPZ 750 combination significantly lowered IL-1β compared with CPZ 750 ($p=0.0337$) and control ($p=0.005$). No significant differences in mmp-3 or IL-1β were observed between SF10 and control. HIF-1α expression was significantly reduced by CPZ, eg, and sf ($p=0.0002$, $p=0.0006$, and $p=0.0063$, respectively). NF-κB1 was downregulated in CPZ- and EG-treated cells ($p=0.0014$ and $p=0.002$, respectively). SF10/CPZ 750 significantly upregulated both HIF-1α and NF-κB1 compared with CPZ ($p=0.0054$ and $p=0.0086$), whereas EG5/CPZ 750 showed no significant change. Data are presented as mean $\pm$ SD; $n=4$

were found between EGz and CPZ, EG and EGz, or control and SF.

HIF-1α – Expression was significantly decreased in CPZ-, EGCG-, and SF-treated cells compared with control ($p=0.0002$, $p=0.0006$, and $p=0.0063$, respectively).

SFz significantly upregulated HIF-1α relative to CPZ ($p=0.0054$) (Fig. 3). No significant changes were detected between EGz and CPZ, EG and EGz, or SF and SFz.

miR-124-3p – Expression was markedly downregulated in CPZ-, SF-, and EGCG-treated cells compared

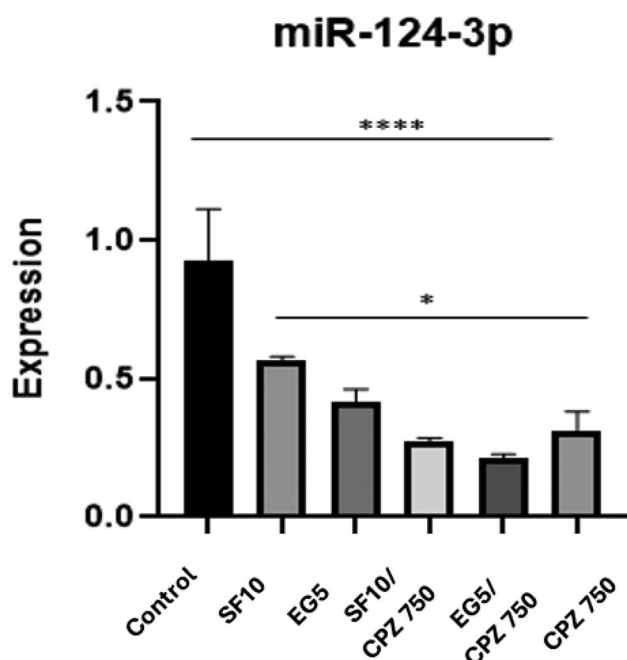


Fig. 4 Quantitative pcr (qRT-PCR) of miR-124-3p quantitative RT-PCR analysis of miR-124-3p expression in SH-SY5Y neuroblastoma cells following cuprizone (CPZ) and extract treatments. miR-124-3p levels were significantly downregulated in CPZ-treated ($p < 0.0001$), green tea extract-treated (eg, $p = 0.0025$), and broccoli extract-treated (sf, $p = 0.0001$) cells compared with control. sf treatment significantly increased miR-124-3p expression relative to CPZ ($p = 0.022$). No significant differences were observed between SF and EG treatments, nor among SF10/ CPZ 750, EG5/ CPZ750, and CPZ 750 groups. Data are presented as mean $\pm$ SD; $n = 4$

to control ($p < 0.0001$, $p = 0.0025$, and $p = 0.0001$, respectively). SF treatment significantly increased miR-124-3p compared to CPZ ($p = 0.022$) (Fig. 4). No significant changes were observed between SF and EG treatments, or among SFz, EGz, and CPZ groups. The amplification plot (Fig. S2) of miR-124-3p, plotted as ΔR_n versus cycle, showed that the endogenous control SNORD38B reached threshold earlier (cycle 22) than miR-124-3p (cycle 29).

CPZ exposure induced marked molecular alterations in SH-SY5Y cells, notably elevating MMP-3, downregulating NF- κ B1, HIF-1 α , and miR-124-3p, while leaving IL-1 β unchanged. Both broccoli (SF) and green tea (EGCG) extracts exerted modulatory effects, with SF showing broader anti-inflammatory and gene-restorative activity—particularly in combination with CPZ—while EGCG demonstrated strong enzymatic suppression. SF + CPZ (SFz) most consistently attenuated CPZ-driven changes, significantly reducing MMP-3 and IL-1 β , and restoring NF- κ B1 and HIF-1 α levels, whereas EGCG + CPZ (EGz) conferred selective protection but also unexpectedly increased MMP-3 compared to EGCG alone.

Collectively, these findings suggest that both extracts can mitigate CPZ-induced neurotoxic signaling through overlapping yet distinct molecular pathways, supporting

their further evaluation as complementary neuroprotective strategies.

Discussion

This study demonstrates that green tea extract (EGCG) and broccoli extract (SF) protect SH-SY5Y neuroblastoma cells from cuprizone (CPZ)-induced cytotoxicity, with optimal efficacy at 5 μ M EGCG and 10 μ M SF. Across MTT, trypan blue exclusion, and microscopy assays, both extracts maintained viability, membrane integrity, and nuclear morphology, with EGCG showing greater acute protection. Although monoculture models cannot fully capture CNS complexity, SH-SY5Y cells retain relevant inflammatory and neuroprotective markers, making them a useful first-line mechanistic platform for screening neuroprotective agents [15, 19].

Phytochemical profiling confirmed catechins and caffeine as the primary bioactive compounds in green tea, and sulforaphane with flavonoids in broccoli. Sulforaphane—a potent Nrf2 activator with high bioavailability—has been shown in vivo to reduce demyelination, inflammation, and blood-brain barrier (BBB) disruption. These bioactive profiles support the observed ability of both extracts to counter CPZ-induced oxidative stress, mitochondrial dysfunction, and inflammatory signaling.

CPZ exposure upregulated MMP-3. EGCG strongly inhibited MMP-3, consistent with its known matrix-stabilizing effects, whereas broccoli extract (SF) preferentially reduced IL-1 β and partially restored miR-124-3p. SF + CPZ broadly normalized inflammatory and survival-associated markers—including MMP-3, IL-1 β , NF- κ B1, and HIF-1 α —indicating a multi-target, pro-resilience profile. EGCG + CPZ provided selective benefits but paradoxically increased MMP-3 relative to EGCG alone, potentially reflecting feedback in matrix remodeling pathways [19] de [15, 16]).

MMP-3 and IL-1 β were both reduced by SF + CPZ, and MMP-3 was strongly suppressed by EGCG. IL-1 β transcription is mediated by NF- κ B [54]. It has been shown that activation of the Nrf2 pathway suppresses IL-1 β expression by binding directly to it [55]. Sulforaphane reduces neuroinflammation via the Nrf2/HO-1 pathway, supporting our findings. Notably, SF uniquely enhanced NF- κ B1 and HIF-1 α expression compared to CPZ, suggesting a broader modulation of inflammatory and survival pathways.

The role of NF- κ B1 in neuroinflammation is cell-type dependent and context specific. For example, NF- κ B1-deficient mice develop normally but resist EAE, potentially via altered T-cell function, whereas neuronal NF- κ B1 inactivation can exacerbate disease [56, 57]. In contrast, Lee et al. [58] reported that NF- κ B ablation in mice showed no difference in the EAE disease progression, suggesting that NF- κ B activity may not be required

for neuroprotection. These contradictory findings may reflect the context-dependent function of NF- κ B1. As highlighted previously [59], NF- κ B can play either pro-apoptotic or neuroprotective roles in the same neuronal cell type depending on the nature of the stimulus. Moreover, NF- κ B1 can promote neuronal survival under stress conditions by enhancing the expression of anti-apoptotic genes such as Bcl-2, and by supporting synaptic plasticity and axon regeneration (Dresselhaus et al., 2019). Furthermore, in oligodendrocytes, NF- κ B1 suppression impairs remyelination in male mice and increases EAE susceptibility in females, suggesting cytoprotective roles under certain inflammatory conditions [58, 60, 61]. Accordingly, our CPZ model revealed NF- κ B1 downregulation following toxin exposure, whereas treatment with broccoli extract restored its expression, suggesting that NF- κ B1 activation may contribute to cytoprotective responses against oxidative and metabolic stress.

Similarly, in neuroinflammation, HIF-1 α has a paradoxical role in addition to its function as the master regulator under hypoxic conditions. It can respond adaptively to cellular stress by promoting glycolytic metabolism, reducing mitochondrial production of reactive oxygen species (ROS), and supporting neuronal survival under toxic conditions. This may explain why HIF-1 α expression was upregulated with SF+CPZ compared to CPZ only. However, HIF-1 α cannot be considered purely neuroprotective because it can enhance the expression of other inflammatory mediators, in addition to being stabilized by IL-1 β and TNF- α under normoxia [62, 63]. Therefore, its upregulation should be considered as an adaptive response to stress and not solely as a neuroprotective signal. Furthermore, it has been reported that HIF-1 α is not necessary for autoimmune demyelination in astrocytes or myeloid cells [35], which again highlights the context-dependent nature of HIF-1 α signaling.

HIF-1 α has been described as neuroprotective in neurons, as it supports survival under hypoxic stress by enhancing glycolysis and overall energy metabolism. It also upregulates neuroprotective factors such as VEGF and erythropoietin (EPO), and attenuates apoptosis triggered by oxidative or metabolic stress, and helps curb excitotoxicity and preserve mitochondrial function. While in Microglia & Astrocytes, it is frequently pro-inflammatory as it drives the transcription of pro-inflammatory cytokines (e.g., IL-1 β , TNF- α), and elevates production of reactive oxygen species (ROS) and nitric oxide (NO). It also contributes to tissue damage, particularly in demyelinating pathologies [64, 65]. This dichotomy underscores a key challenge for intervention: targeting HIF-1 α in MS or other demyelinating diseases requires precision approaches that amplify its neuroprotective programs in neurons while restraining its inflammatory signaling in glial cells. Such cell-type-specific

modulation could enhance tissue preservation without inadvertently worsening inflammation.

miR-124-3p, typically low in neurons but rising during neurogenesis, supports neuronal survival, suppresses apoptosis, and improves recovery after hypoxic injury [32]. It is downregulated in EAE and other neurodegeneration models [31]. Here, CPZ markedly reduced miR-124-3p, consistent with these reports. While some phytochemicals such as resveratrol can restore miR-124-3p and alleviate disease [66], neither broccoli nor green tea significantly affected its expression in this study—possibly due to the low concentrations used or model-specific factors.

CPZ exposure produced a pro-degenerative molecular signature—elevating MMP-3, suppressing NF- κ B1 and HIF-1 α , and downregulating miR-124-3p—while leaving IL-1 β unchanged. Both extracts mitigated elements of this response via distinct yet overlapping mechanisms: EGCG acted predominantly on matrix degradation pathways, while SF demonstrated broader anti-inflammatory and gene-restorative effects, particularly in combination with CPZ. These complementary profiles suggest that broccoli and green tea extracts merit further investigation as adjunct or combinational strategies for mitigating demyelination-associated neurotoxicity in MS and related disorders.

Given the translational relevance of CPZ as a demyelination model, these findings have potential implications for neurodegenerative conditions such as multiple sclerosis, where oxidative stress, inflammation, and matrix remodeling converge. Future studies should investigate the downstream signaling pathways—particularly Nrf2, NF- κ B, and hypoxia-responsive elements—that mediate these protective effects, and assess whether combined or staged extract administration offers additive or synergistic benefits. In vivo validation and pharmacokinetic profiling will also be critical to determine therapeutic feasibility.

Limitations

This study represents an in vitro proof-of-concept, with several limitations that define the scope of its conclusions and point toward future investigative needs. The SH-SY5Y neuroblastoma cell line, while widely used in neurotoxicity research, cannot fully reproduce the cellular diversity, myelination, and microenvironment of the human CNS, thereby limiting direct translational applicability. Furthermore, CPZ served as the sole inducer of injury, capturing oxidative and inflammatory aspects of demyelination but not the autoimmune or multifactorial mechanisms characteristic of disorders such as multiple sclerosis.

Although multiple molecular targets were profiled, upstream signaling pathways—such as Nrf2 activation,

MAPK cascades, and apoptotic regulators—were not directly examined, leaving gaps in mechanistic understanding. The experimental design also tested only two concentrations of each extract over a single 24-hour exposure, which restricts insight into optimal dosing, temporal dynamics, and potential biphasic effects. While GC–MS identified key bioactive constituents, the relative contributions of individual compounds, as well as possible synergistic or antagonistic interactions, remain unresolved.

To address these limitations, future studies should incorporate more complex in vitro systems, such as co-cultures of neuronal cells and oligodendrocytes, alongside in vivo models and eventual clinical investigations. Pharmacokinetic analyses will be essential to determine bioavailability and blood–brain barrier penetration. Additionally, mapping both upstream and downstream signaling networks—including oxidative stress responses, inflammatory transcription factors, and apoptosis pathways—will help pinpoint mechanistic drivers and refine extract-based neuroprotective strategies.

Supplementary information

The online version contains supplementary material available at <https://doi.org/10.1186/s40816-025-00407-z>.

Supplementary Material 1

Author contributions

S.F. performed the experiments, prepared figures, and wrote the manuscript draft. A.A. supervised the work and edited and revised the manuscript. All authors approved the manuscript before submission.

Funding

This work was supported by a research grant from the American University in Cairo.

Data availability

All data generated is included in the manuscript and supplementary material.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 28 May 2025 / Accepted: 28 October 2025

Published online: 07 November 2025

References

- Chirchiù V, Stelt M, Centonze D, Maccarrone M. The Endocannabinoid system and its Therapeutic exploitation in multiple sclerosis: clues for other neuroinflammatory diseases. *Prog In Neurobiol*. 2017, October;160. <https://doi.org/10.1016/j.pneurobio.2017.10.007>
- Ghasemi N, Razavi S, Nikzad E. Multiple sclerosis: pathogenesis, symptoms, diagnoses and cell-based therapy. *Cell J (yakhteh)*. 2017;19(1):1
- Strober LB. Fatigue in multiple sclerosis: a look at the role of poor sleep. *Front Neurol*. 2015;6(February):21. <https://doi.org/10.3389/fneur.2015.00021>
- Sand K, I. The role of diet in multiple sclerosis: mechanistic connections and current evidence. *Curr Nutr Rep*. 2018;7(3):150–60. <https://doi.org/10.1007/s13668-018-0236-z>
- Mulero P, Midaglia L, Montalban X. Ocrelizumab: a new milestone in multiple sclerosis therapy. *Ther Adv Neurol Disord*. 2018;11(May):1756286418773025. <https://doi.org/10.1177/1756286418773025>
- Aly L, Hemmer B, Korn T. From Leflunomide to Teriflunomide: drug development and immuno-suppressive oral drugs in the treatment of multiple sclerosis. *Curr Neuropharmacol*. 2017;15(6):874–91. <https://doi.org/10.2174/1570159X14666161208151525>
- Lamb YN. Ocrelizumab: a review in multiple sclerosis. *Drugs*. 2022;82(3):323–34. <https://doi.org/10.1007/s40265-022-01672-9>
- Dolati S, Ahmadi M, Aghebti-Maleki L, et al. Nanocurcumin is a potential novel therapy for multiple sclerosis by influencing inflammatory mediators. *Pharmacological Rep: Pr*. 2018;70(6):1158–67. <https://doi.org/10.1016/j.pharep.2018.05.008>
- Theodosios-Nobelos P, Rekka EA. The multiple sclerosis modulatory potential of natural multi-targeting antioxidants. *Mol (basel, Switzerland)*. 2022;27(23):8402. <https://doi.org/10.3390/molecules27238402>
- Ladak Z, Garcia E, Yoon J, et al. Sulforaphane (SFA) protects neuronal cells from oxygen & glucose deprivation (OGD). *PLoS One*. 2021;16(3):3. <https://doi.org/10.1371/journal.pone.0248777>
- Farooqui T, Akhlaq AF. Chapter 16 - neuroprotective effects of garlic in model systems of neurodegenerative diseases. In: Farooqui T, Farooqui AA, editors. *Role of the Mediterranean Diet in the brain and neurodegenerative diseases*. Academic Press; 2018. <https://doi.org/10.1016/B978-0-12-811959-4.00016-X>
- Sun Q, Zheng Y, Zhang X, et al. Novel immunoregulatory properties of EGCG on reducing inflammation in EAE. *Front Biosci (landmark Ed)*. 2013;18(January):332–42. <https://doi.org/10.2741/4104>
- Peng Y, Chu S, Yang Y, Zhang Z, Pang Z, Chen N. Neuroinflammatory in vitro cell culture models and the potential applications for neurological disorders. *Front Pharmacol*. 2021, April;12. <https://doi.org/10.3389/fphar.2021.671734>
- Martínez-Pinilla E, Rubio-Sardón N, Peláez R, et al. Neuroprotective effect of apolipoprotein D in Cuprizone-induced cell line models: a potential therapeutic approach for multiple sclerosis and demyelinating diseases. *Int J Mol Sci*. 2021;22(3):3. <https://doi.org/10.3390/ijms22031260>
- Lopez-Suarez L, Al Awabdh S, Coumoul X, Chauvet C. The SH-SY5Y human neuroblastoma cell line, a relevant in vitro cell model for investigating neurotoxicology in human: focus on organic pollutants. *Neurotoxicology*. 2022;92(September):131–55. <https://doi.org/10.1016/j.neuro.2022.07.008>
- Medeiros LMD, De Bastiani MA, Rico EP, et al. Cholinergic differentiation of human neuroblastoma SH-SY5Y cell line and its potential use as an in vitro Model for Alzheimer's disease studies. *Mol Neurobiol*. 2019;56(11):7355–67. <https://doi.org/10.1007/s12035-019-1605-3>
- Agholme L, Lindström T, Kågedal K, Marcusson J, Hallbeck M. An in vitro model for neuroscience: differentiation of SH-SY5Y cells into cells with morphological and biochemical characteristics of mature neurons. *J Educ Chang Alzheimer's Disease: JAD*. 2010;20(4):1069–82. <https://doi.org/10.3233/JAD-2010-091363>
- D'Aloia A, Pastori V, Blasa S, et al. A new advanced cellular Model of functional cholinergic-like neurons developed by reprogramming the human SH-SY5Y neuroblastoma cell line. *Cell Death Discov*. 2024;10(1):24. <https://doi.org/10.1038/s41420-023-01790-7>
- Kovalevich J, Langford D. Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. *Methods In Mol Biol (Clifton, N.J.)*. 2013;1078:9–21. https://doi.org/10.1007/978-1-62703-640-5_2
- Martínez-Pinilla E, Rubio-Sardón N, Villar-Conde S, et al. Cuprizone-induced neurotoxicity in human neural cell lines is mediated by a reversible mitochondrial dysfunction: relevance for demyelination models. *Brain Sci*. 2021;11(2):2. <https://doi.org/10.3390/brainsci11020272>
- Bénardais K, Kotsiari A, Škuljec J, et al. Cuprizone [Bis(Cyclohexylidenehydrazide)] is selectively toxic for mature oligodendrocytes. *Neurotox Res*. 2013;24(2):244–50. <https://doi.org/10.1007/s12640-013-9380-9>
- Zirngibl M, Assinck P, Sizov A, Capriarello AV, Plemel JR. Oligodendrocyte death and myelin loss in the Cuprizone model: an updated overview of the intrinsic and extrinsic causes of Cuprizone demyelination. *Mol Neurodegener*. 2022;17(1):34. <https://doi.org/10.1186/s13024-022-00538-8>

23. Benetti F, Ventura M, Salmini B, et al. Cuprizone neurotoxicity, copper deficiency and neurodegeneration. *Neurotoxicol, Gene-Environ Interact*. In *Neurotoxicol: The 12th Bienn Meet of The Int Neurotoxicol Assoc*. 2010;31(5):509–17. <https://doi.org/10.1016/j.neuro.2010.05.008>
24. Bénardais K, Kotsiari A, Skuljec J, et al. Cuprizone [Bis(Cyclohexylidenehydrazide)] Is Selectively Toxic for Mature Oligodendrocytes. *Neurotox Res*. 2013;24(2):2. <https://doi.org/10.1007/s12640-013-9380-9>
25. Taraboletti A, Walker T, Avila R, et al. Cuprizone intoxication induces cell intrinsic alterations in oligodendrocyte metabolism Independent of copper chelation. *Biochemistry*. 2017;56(10):10. <https://doi.org/10.1021/acs.biochem.6b01072>
26. Celik ZB, Nihan Cankara F, Gunaydin C. Alterations in the matrix Metalloproteinase-3 promoter methylation after common chemotherapeutics: In Vitro study of Paclitaxel, cisplatin and methotrexate in the MCF-7 and SH-SY5Y cell lines. *Mol Biol Rep*. 2020;47(11):11. <https://doi.org/10.1007/s11033-020-0595-5-w>
27. Chih-Chung L, Edelson BT. New insights into the role of IL-1 β in EAE and MS. *The J Immunol*. 2017;198(12):12. <https://doi.org/10.4049/jimmunol.1700263>
28. Heidary M, Rakhshi N, Kakhki MP, et al. The analysis of correlation between IL-1B gene expression and genotyping in multiple sclerosis patients. *J Neurological Sci*. 2014;343(1–2):1–2. <https://doi.org/10.1016/j.jns.2014.05.013>
29. Seppi D, Puthenparampil M, Federle L, et al. Cerebrospinal fluid IL-1 β correlates with cortical pathology load in multiple sclerosis at clinical onset. *J Neuroimmunol*. 2014;270(1–2):1–2. <https://doi.org/10.1016/j.jneuroim.2014.02.014>
30. Hoyos HC, Rinaldi M, Mendez-Huergo SP, et al. Galectin-3 controls the response of microglial cells to limit Cuprizone-induced demyelination. *Neurobiol Dis*. 2014;62(February):441–55. <https://doi.org/10.1016/j.nbd.2013.10.023>
31. Gao Y, Han D, Feng J. MicroRNA in multiple sclerosis. *Clinica (Chimica) Acta*. 2021;516(May):92–99. <https://doi.org/10.1016/j.cca.2021.01.020>
32. Xiong L, Zhou H, Zhao Q, et al. Overexpression of miR-124 protects against neurological dysfunction induced by neonatal hypoxic–ischemic brain injury. *Cellular Mol Neurobiol*. 2020;40(5):5. <https://doi.org/10.1007/s10571-019-00769-2>
33. Kanwar J, Taskeen M, Mohammad I, Huo C, Hang Chan T, Ping Dou Q. Recent advances on tea polyphenols. *Front Biosci (Elite Ed)*. 2012;4(January):111–31
34. Luo J-L, Kamata H, Karin M. IKK/NF- κ B signaling: balancing life and death – a new approach to cancer therapy. *J. Clin. Invest*. 2005;115(10):10. <https://doi.org/10.1172/JCI26322>
35. Moan L, Natacha KMB, Rafalski VA, et al. Hypoxia inducible factor-1 α in astrocytes and/or myeloid cells is not required for the development of autoimmune demyelinating disease. *eNeuro*. 2015;2(2):2. <https://doi.org/10.1523/ENEURO.0050-14.2015>
36. Saravani M, Rokni M, Mehrbani M, et al. The evaluation of VEGF and HIF-1 α gene polymorphisms and multiple sclerosis susceptibility. *J Gene Med*. 2019;21(12):12. <https://doi.org/10.1002/jgm.3132>
37. Ristori E, Stefania N. Chapter 15 - comparative functions of miRNAs in embryonic neurogenesis and neuronal network formation. In: Tonelli DDP, editor. *Essentials of noncoding rna in neuroscience*. Academic Press; 2017. <https://doi.org/10.1016/B978-0-12-804402-5.00015-7>
38. Li R, Zhu Y. Encapsulation of sulforaphane from cruciferous vegetables in mPEG-PLGA nanoparticles enhances Cadmium's inhibitory effect on HepG2 cells. *Nanomaterials*. 2025;15(8):615. <https://doi.org/10.3390/nano15080615>
39. Shariare H, Mohammad KA, Iqbal F, et al. Development and optimization of epigallocatechin-3-Gallate (EGCG) Nano phytosome using design of experiment (DoE) and their in vivo anti-inflammatory studies. *Mol (basel, Switzerland)*. 2020;25(22):5453. <https://doi.org/10.3390/molecules25225453>
40. Wesołowska A, Jadcak D, Grzeszczuk M. Chemical composition of the pepper fruit extracts of hot cultivars capsicum annum L *Acta Sci Pol Hortorum Cultus*. 2011;10(January):171–84
41. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta C(T)) method. *Methods (san Diego, Calif)*. 2001;25(4):402–08. <https://doi.org/10.1006/meth.2001.1262>
42. Hae-Suk K, Quon MJ, Kim J-A. New insights into the mechanisms of polyphenols beyond antioxidant Properties; lessons from the Green tea polyphenol, epigallocatechin 3-gallate. *Redox Biol*. 2014;2(January):187–95. <https://doi.org/10.1016/j.redox.2013.12.022>
43. De La Cruz JP, Ruiz-Moreno MI, Guerrero A, et al. Role of the catechol group in the antioxidant and Neuroprotective effects of Virgin Olive oil components in rat brain. *J Nutritional Biochem*. 2015;26(5):5. <https://doi.org/10.1016/j.jnutbio.2014.12.013>
44. Xie X, Wu F, Tian J, et al. Pyrocatechol alleviates cisplatin-induced acute kidney injury by inhibiting ros production. *Oxidative Med Cell Longev*. 2022;2022:2158644. <https://doi.org/10.1155/2022/2158644>
45. Yashin A, Yashin Y, Yuan Wang J, Nemzer B. Antioxidant and antiradical activity of coffee. *Antioxid (basel, Switz)*. 2013;2(4):4. <https://doi.org/10.3390/antiox2042030>
46. Schepici G, Bramanti P, Mazzon E. Efficacy of sulforaphane in neurodegenerative diseases. *Int J Mol Sci*. 2020;21(22):8637. <https://doi.org/10.3390/ijms21228637>
47. Pinto MEA, Araújo SG, Morais MI, et al. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. *An Acad Bras Cienc*. 2017;89(August):1671–81. <https://doi.org/10.1590/0001-3765201720160908>
48. Uchegbu NN, Omolayo Fasuan T, Love Onuoha N. Quantification of Phytochemicals, and compounds' identification in functional tea from *Ficus Capensis* and *Justicia Secunda*. *J Food Sci*. 2023;88(3):1004–18. <https://doi.org/10.1111/1750-3841.16459>
49. Zhang Y, Xiang Z, Xu Y, et al. Oleic acid restores the impaired antitumor immunity of T_H1 cells induced by palmitic acid. *Signal Transduct And Targeted Ther*. 2025;10(1):209. <https://doi.org/10.1038/s41392-025-02295-8>
50. Budzynska B, Faggio C, Kruk-Slomka M, et al. Rutin as neuroprotective agent: from bench to bedside. *Curr Med Chem*. 2019;26(27):27. <https://doi.org/10.2174/0929867324666171003114154>
51. Enogieru AB, Haylett W, Charles Hiss D, Bardien S, Eko Ekpo O. Rutin as a potent antioxidant: implications for neurodegenerative disorders. *Oxidative Med Cell Longev*. 2018 2018, June:6241017. <https://doi.org/10.1155/2018/6241017>
52. Lee H-C, Reggie ACE, Silva HEP, et al. Palmitic acid methyl ester is a novel Neuroprotective agent against cardiac arrest. *Prostaglandins Leukot Essent Fat Acids*. 2019;147(August):6–14. <https://doi.org/10.1016/j.plefa.2018.11.011>
53. Baskar AA, Al Numair KS, Gabriel Paulraj M, Alsaif MA, Al Muamar M, Ignacimuthu S. β -sitosterol prevents lipid peroxidation and improves antioxidant status and histoarchitecture in rats with 1,2-dimethylhydrazine-induced colon cancer. *J Med Food*. 2012;15(4):4. <https://doi.org/10.1089/jmf.2011.1780>
54. Subedi L, Hyuk Lee J, Yumnam S, Ji E, Yeou Kim S. Anti-inflammatory effect of sulforaphane on LPS-Activated microglia potentially through JNK/AP-1/NF- κ B inhibition and Nrf2/HO-1 activation. *Cells*. 2019;8(2):194. <https://doi.org/10.3390/cells8020194>
55. Wall SB, Li R, Butler B, et al. Auranofin-mediated NRF2 induction attenuates interleukin 1 beta expression in alveolar macrophages. *Antioxid (Basel, Switzerland)*. 2021;10(5):632. <https://doi.org/10.3390/antiox10050632>
56. Emmanouil M, Taoufik E, Tseveleki V, et al. Neuronal I κ B kinase β protects mice from autoimmune encephalomyelitis by mediating neuroprotective and immunosuppressive effects in the central nervous system. *The J Immunol*. 2009;183(12):12. <https://doi.org/10.4049/jimmunol.0900834>
57. Zhou Z, Xiong H, Xie F, Wu Z, Feng Y. A meta-analytic review of the value of miRNA for multiple sclerosis diagnosis. *Front Neurol*. 2020;11:132. <https://doi.org/10.3389/fneur.2020.00132>
58. Lee D-H, Kubera K, Rosenthal B, et al. Neuronal NF- κ B ablation does not influence Neuro-axonal degeneration in experimental autoimmune demyelination. *J Neuroimmunol*. 2012;246(1):38–42. <https://doi.org/10.1016/j.jneuroim.2012.03.005>
59. Kaltschmidt B, Heinrich M, Kaltschmidt C. Stimulus-dependent activation of NF- κ B specifies apoptosis or neuroprotection in cerebellar granule cells. *NeuroMol Med*. 2002;2(3):299–309. <https://doi.org/10.1385/NMMD.2.3.299>
60. Dresselhaus EC, Meffert MK. Cellular specificity of NF- κ B function in the nervous system. *Front Immunol*. 2019, May;10. <https://doi.org/10.3389/fimmu.2019.01043>
61. Guire M, Conon MP, Beyaert R, van Loo G. Nuclear factor kappa B (NF- κ B) in multiple sclerosis pathology. *Trends Mol Med*. 2013;19(10):10. <https://doi.org/10.1016/j.molmed.2013.08.001>
62. Asgari R, Yarani R, Mohammadi P, Sajad Emami Aleagha M. HIF-1 α in the crosstalk between reactive oxygen species and autophagy process: a review in multiple sclerosis. *Cellular Mol Neurobiol*. 2022;42(7):2121–29. <https://doi.org/10.1007/s10571-021-01111-5>
63. Soroush A, Dunn JF. A hypoxia-inflammation cycle and multiple sclerosis: mechanisms and therapeutic implications. *Curr Treat Options Neurol*. 2024;27(1):6. <https://doi.org/10.1007/s11940-024-00816-4>
64. Choi YK. Detrimental roles of hypoxia-inducible factor-1 α in severe hypoxic brain diseases. *Int J Mol Sci*. 2024;25(8):4465. <https://doi.org/10.3390/ijms25084465>

65. Mitroshina EV, Vedunova MV. The role of oxygen homeostasis and the HIF-1 Factor in the development of neurodegeneration. *Int J Mol Sci.* 2024;25(9):4581. <https://doi.org/10.3390/ijms25094581>
66. Gandy KAO, Zhang J, Nagarkatti P, Nagarkatti M. Resveratrol (3, 5, 4'-trihydroxy-trans-stilbene) attenuates a mouse Model of multiple sclerosis by altering the miR-124/Sphingosine kinase 1 axis in encephalitogenic T cells in the brain. *J Neuroimmune Pharmacol.* 2019;14(3):3. <https://doi.org/10.1007/s11481-019-09842-5>

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.