**RESEARCH PAPER**

# Quercetin Attenuates Glutamate-Induced Oxidative Stress and Restores Antioxidant Defence in SH-SY5Y Neuroblastoma Cells

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**ABSTRACT**

**Background:** Glutamate-induced excitotoxicity and oxidative stress are central mechanisms underlying neuronal injury and the progression of neurodegenerative diseases. Quercetin, a dietary flavonoid, is recognized for its potent antioxidant and cytoprotective properties. This study investigated the neuroprotective effects of quercetin against glutamate-induced cytotoxicity in SH-SY5Y neuroblastoma cells.

**Methods:** SH-SY5Y cells were pretreated with quercetin (5–20 μM) prior to glutamate (10 mM) exposure. Cell viability was evaluated using the MTT assay. Oxidative stress markers were assessed through intracellular ROS measurement, LDH release, antioxidant enzyme activities (SOD, CAT, GSH), lipid peroxidation (MDA), and nitric oxide (NO) production.

**Results:** Glutamate significantly reduced cell viability and induced oxidative/nitrosative stress, as evidenced by elevated ROS, LDH, MDA, and NO levels alongside suppressed antioxidant enzyme activities. Quercetin pretreatment dose-dependently restored cell survival (up to 89.5%), suppressed ROS accumulation, decreased LDH leakage, lowered MDA and NO levels, and significantly enhanced SOD, CAT, and GSH activities.

**Conclusion:** Quercetin effectively protects SH-SY5Y cells from glutamate-induced excitotoxicity by reducing oxidative damage, preserving membrane integrity, and reinforcing antioxidant defenses. These findings highlight quercetin's potential as a therapeutic candidate for managing oxidative stress–driven neurodegenerative disorders.

**Keywords:** Quercetin, SH-SY5Y cells, Glutamate excitotoxicity, Oxidative stress, LDH release, Antioxidant enzymes, Lipid peroxidation, Nitric oxide.

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## Introduction

Neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD) are devastating conditions marked by progressive neuronal loss, synaptic dysfunction, and gradual cognitive and motor decline (Przedborski et al., 2003; Dugger & Dickson, 2017). With aging populations worldwide, their prevalence is increasing rapidly, imposing a substantial global health burden (GBD 2019 Dementia Forecasting Collaborators, 2022). Despite decades of research, current treatments only offer symptomatic relief and fail to halt disease progression (Kumar et al., 2015). Among the complex mechanisms involved, oxidative stress and glutamate-mediated excitotoxicity are recognized as central drivers of neuronal injury (Wang & Michaelis, 2010).

Excitotoxicity arises from excessive activation of glutamate receptors, particularly NMDA receptors, leading to uncontrolled calcium influx, enzyme activation, mitochondrial dysfunction, and increased reactive oxygen species (ROS) production (Dong et al., 2009). Under physiological conditions, glutamate is essential for learning, memory, and synaptic plasticity (Meldrum, 2000). However, pathological elevations in glutamate create a toxic environment that damages proteins, lipids, and nucleic acids, often culminating in neuronal apoptosis or necrosis (Lewerenz & Maher, 2015). The interplay between excitotoxicity and oxidative stress generates a vicious cycle in which ROS accumulation further impairs mitochondria, amplifying neuronal damage (Nicholls, 2009). Targeting this cycle has thus become a promising strategy for neuroprotection (Coyle & Puttfarcken, 1993). The transcription factor nuclear factor erythroid 2–related factor 2 (Nrf2) is a central regulator of antioxidant defense. Under stress, Nrf2 translocates to the nucleus and activates antioxidant response element (ARE)-driven genes such as heme oxygenase-1 (HO-1), NAD(P)H quinone oxidoreductase-1 (NQO1), and glutamate-cysteine ligase (GCL), which protect cells from oxidative injury (Kensler et al., 2007; Ma, 2013). Impaired Nrf2 signaling has been linked to progression of neurodegenerative disorders, while pharmacological or dietary activators of Nrf2 confer cytoprotection in experimental models (Johnson et al., 2008; Sandberg et al., 2014).

Natural compounds have gained attention for their ability to modulate oxidative stress and neuronal survival pathways. Among these, quercetin, a dietary flavonoid abundant in fruits, vegetables, tea, and wine, has been extensively studied for its antioxidant and anti-inflammatory properties. Its polyphenolic structure with multiple hydroxyl groups confers potent radical-scavenging activity (Boots et al., 2008). Beyond direct antioxidant effects, quercetin modulates signaling cascades such as MAPK, PI3K/Akt, and Nrf2/HO-1, thereby enhancing stress resistance and neuroprotection (Dajas, 2012; Khan et al., 2020). Previous studies have shown quercetin protects neurons against ischemic injury, toxin-induced oxidative stress, and age-related neurodegeneration (Ishige et al., 2001; Wang et al., 2011). Despite these promising findings, the precise impact of quercetin on glutamate-induced excitotoxicity in human-derived neuronal models remains underexplored. SH-SY5Y neuroblastoma cells provide a well-established in vitro system for studying neuronal physiology and excitotoxic injury (Xicoy et al., 2017). Establishing glutamate-induced damage in SH-SY5Y cells enables evaluation of candidate compounds such as quercetin under controlled conditions.

In this context, the present study aimed to systematically evaluate quercetin's neuroprotective potential against glutamate-induced toxicity in SH-SY5Y cells. Specifically, the objectives were: (1) to establish glutamate-induced oxidative stress and cytotoxicity in SH-SY5Y cells; (2) to assess quercetin's protective effect on cell viability; (3) to examine oxidative stress parameters, including ROS production, LDH release, antioxidant enzyme activities (SOD, CAT, GSH), lipid peroxidation (MDA), and nitric oxide (NO) levels; and (4) to interpret how these effects reflect quercetin's broader role as a neuroprotective antioxidant. By integrating viability assays and multiple biochemical endpoints, this investigation provides a comprehensive evaluation of quercetin's ability to suppress excitotoxic injury and restore neuronal redox balance. Insights gained may support the future development of quercetin or its optimized derivatives as therapeutic interventions for oxidative stress–related neurodegenerative disorders.

## Materials and Methods

### Cell Line and Culture

Human neuroblastoma SH-SY5Y cells were obtained from the National Centre for Cell Science (NCCS), Pune, India. These cells are widely accepted as an in vitro neuronal model owing to their catecholaminergic nature and ability to mimic dopaminergic neurons after differentiation (Xicoy et al., 2017). Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, high glucose formulation; Gibco, USA) supplemented

with 10% fetal bovine serum (FBS; Invitrogen, USA) to provide essential nutrients and growth factors. Penicillin (100 U/ml) and streptomycin (100 µg/ml) were included to prevent microbial contamination (Freshney, 2010). Cultures were maintained at 37 °C in a humidified incubator with 95% air and 5% $CO_2$. The medium was changed every 2–3 days to maintain nutrient balance. Cells were subcultured at 70–80% confluence using 0.25% trypsin-EDTA. Only cells between passages 5–15 were used to maintain phenotypic stability (Agholme et al., 2010). All manipulations were performed aseptically in a Class II biosafety cabinet (Esco, Singapore).

**Chemicals and Reagents**

Quercetin (≥95% purity, Sigma-Aldrich, St. Louis, MO, USA) was dissolved in dimethyl sulfoxide (DMSO) and diluted in culture medium to working concentrations. The final DMSO concentration in the medium was kept below 0.1% to avoid solvent-induced toxicity (Mosmann, 1983). L-glutamate (Sigma-Aldrich) was freshly prepared in sterile phosphate-buffered saline (PBS) before use to minimize degradation. Other reagents included MTT for cell viability (Mosmann, 1983), DCFH-DA for intracellular ROS detection (Eruslanov & Kusmartsev, 2010), and LDH assay reagents (Cayman Chemical, USA). Kits for antioxidant enzyme assays (SOD, CAT, GSH) and lipid peroxidation (MDA) were procured from Cayman Chemical (USA). Nitric oxide levels were quantified using Griess reagent. All other chemicals and solvents used were of analytical grade (Merck, Germany).

**Experimental Design**

Cells were grouped as follows:

1. **Control group:** Untreated SH-SY5Y cells.
2. **Glutamate group:** Exposure to 10 mM glutamate for 24 h to induce excitotoxicity and oxidative stress (Frandsen & Schousboe, 1993).
3. **Quercetin alone group:** Cells treated with 20 µM quercetin without glutamate exposure to verify non-toxicity.
4. **Quercetin + Glutamate group:** Cells pretreated with quercetin (5, 10, and 20 µM) for 2 h, followed by glutamate (10 mM, 24 h). Pretreatment allowed intracellular accumulation of quercetin and simulated prophylactic protection (Dajas, 2012).

All experiments were performed in triplicate biological replicates, with technical triplicates included.

**Biochemical Evaluations**

**Cell Viability (MTT Assay)**

Cell viability was measured by the MTT assay, which detects mitochondrial metabolic activity. SH-SY5Y cells were seeded at $1 \times 10^4$ cells/well in 96-well plates and allowed to adhere overnight. After treatments, 20 µl of MTT (5 mg/ml in PBS) was added per well and incubated for 4 h at 37 °C in the dark. Mitochondrial dehydrogenases in viable cells reduced MTT to purple formazan crystals, which were dissolved in 150 µl of DMSO. Absorbance was read at 570 nm using a microplate reader (BioTek Synergy HT, USA). Cell viability was expressed as percentage relative to untreated controls (Mosmann, 1983).

**Intracellular ROS Measurement**

ROS levels were quantified using DCFH-DA, a non-fluorescent probe that is oxidized to fluorescent dichlorofluorescein (DCF) by ROS. Cells were seeded at $1 \times 10^5$/well in 6-well plates, treated, washed with PBS, and incubated with 10 µM DCFH-DA for 30 min at 37 °C in the dark. Excess dye was removed, and fluorescence was measured at 485/530 nm using a spectrofluorometer (PerkinElmer LS55, USA). Results were expressed relative to controls. $H_2O_2$-treated cells served as positive controls (Eruslanov & Kusmartsev, 2010).

**Lactate Dehydrogenase (LDH) Release**

LDH release into the culture medium, reflecting plasma membrane damage, was quantified using a commercial kit (Cayman Chemical, USA). After treatments, supernatants were collected and analyzed according to the manufacturer's protocol. Absorbance was measured at 490 nm, and cytotoxicity was expressed as a percentage relative to control.

**Antioxidant Enzyme Assays**

- **Superoxide Dismutase (SOD):** Activity was assessed by the ability of SOD to inhibit pyrogallol autoxidation, with absorbance monitored at 420 nm. Enzyme activity was expressed as U/mg protein (Marklund & Marklund, 1974).
- **Catalase (CAT):** Activity was determined by measuring the decomposition of hydrogen peroxide at 240 nm, expressed as U/mg protein (Aebi, 1984).
- **Reduced Glutathione (GSH):** Levels were estimated using Ellman's reagent (DTNB), which reacts with sulfhydryl groups to form a yellow chromophore measurable at 412 nm. Results were expressed as µmol/mg protein (Ellman, 1959).

**Lipid Peroxidation (MDA Levels)**

Malondialdehyde (MDA), an index of lipid peroxidation, was quantified by the thiobarbituric acid reactive substances (TBARS) method. Cell lysates were reacted with thiobarbituric acid, and the resulting pink chromogen was measured at 532 nm. MDA levels were expressed as nmol/mg protein (Ohkawa et al., 1979).

**Nitric Oxide (NO) Production**

Nitric oxide production was determined using the Griess reaction. Culture supernatants were mixed with Griess reagent (sulfanilamide and NED), producing a pink azo dye measurable at 540 nm. Nitrite concentration, a stable NO metabolite, was calculated from a sodium nitrite standard curve and expressed as µM (Green et al., 1982).

**Protein Estimation**

Protein concentrations in cell lysates were measured by the Bradford method using bovine serum albumin as a standard (Bradford, 1976). Results were used for normalization of enzyme activities and oxidative stress parameters.

**Statistical Analysis**

All experiments were carried out in triplicate (n = 3). Data were expressed as mean ± SD. One-way ANOVA followed by Tukey's post hoc test was used for statistical comparisons. A p-value < 0.05 was considered significant. Graphs were prepared using GraphPad Prism version 9 (GraphPad Software, 2020).

**Results**

**Effect of Quercetin on Cell Viability**

Glutamate exposure caused a pronounced decline in SH-SY5Y cell viability, reducing it to 48.6 ± 3.2% compared with 100.0 ± 2.5% in the control group, confirming its excitotoxic and cytotoxic nature. Quercetin pretreatment significantly restored viability in a concentration-dependent manner. At 5 µM, viability improved to 62.3 ± 2.8%, while 10 µM and 20 µM further enhanced survival to 74.8 ± 3.1% and 89.5 ± 2.6%, respectively. Importantly, quercetin alone at 20 µM showed no cytotoxicity, maintaining cell viability at 98.1 ± 2.2%, comparable to control. These findings indicate that quercetin counteracts glutamate-induced cell death and provides a strong neuroprotective effect, especially at higher concentrations.

**Table 1.** Effect of Quercetin on SH-SY5Y Cell Viability (MTT Assay)

| Treatment Group | Cell Viability (%) |
|---|---|
| Control | 100.0 ± 2.5 |
| Glutamate (10 mM) | 48.6 ± 3.2 |
| Quercetin (5 µM) + Glutamate | 62.3 ± 2.8 |
| Quercetin (10 µM) + Glutamate | 74.8 ± 3.1 |
| Quercetin (20 µM) + Glutamate | 89.5 ± 2.6 |
| Quercetin alone (20 µM) | 98.1 ± 2.2 |

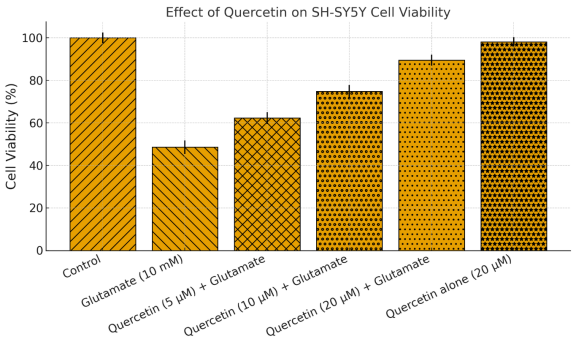


**Figure 1.** Effect of quercetin pretreatment on SH-SY5Y cell viability under glutamate-induced stress.

**Effect of Quercetin on Intracellular ROS Generation**

Intracellular ROS levels increased sharply following glutamate exposure, reaching 245.3 ± 8.3% of control, confirming oxidative stress as a key mechanism of glutamate toxicity. Quercetin pretreatment markedly attenuated ROS generation in a dose-dependent manner. At 5 µM, ROS decreased to 198.6 ± 7.2%, while 10 µM and 20 µM reduced levels further to 152.7 ± 6.8% and 118.4 ± 5.9%, respectively. Quercetin alone slightly lowered baseline ROS levels to 96.7 ± 4.7%, suggesting it exerts an antioxidant effect even under non-stress conditions. These results highlight quercetin's efficiency in counteracting glutamate-induced oxidative stress by limiting excessive ROS accumulation, thereby protecting neuronal cells from oxidative damage.

**Table 2.** Effect of Quercetin on Intracellular ROS Levels in SH-SY5Y Cells

| Treatment Group | ROS Level (% of Control) |
|---|---|
| Control | 100.0 ± 5.4 |
| Glutamate (10 mM) | 245.3 ± 8.3 |
| Quercetin (5 µM) + Glutamate | 198.6 ± 7.2 |
| Quercetin (10 µM) + Glutamate | 152.7 ± 6.8 |
| Quercetin (20 µM) + Glutamate | 118.4 ± 5.9 |

| | |
|---|---|
| Quercetin alone (20 µM) | 96.7 ± 4.7 |

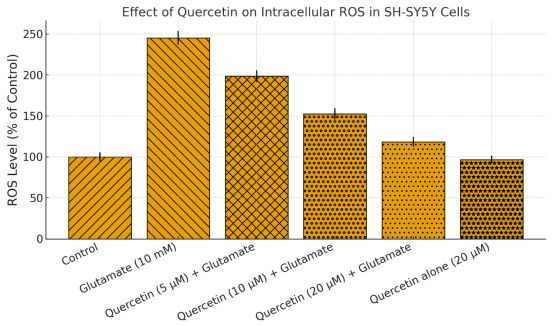


**Figure 2.** Effect of quercetin on intracellular ROS generation in SH-SY5Y cells.

**Effect of Quercetin on LDH Release**

Glutamate exposure resulted in a substantial increase in LDH release (235.6 ± 9.2% of control), reflecting severe loss of membrane integrity and cell lysis. Quercetin pretreatment significantly suppressed LDH leakage in a concentration-dependent fashion. At 5 µM, LDH levels were reduced to 185.4 ± 8.1%, while at 10 µM and 20 µM they declined further to 142.3 ± 7.5% and 112.6 ± 6.8%, respectively. Quercetin alone maintained LDH values at near-control levels (98.9 ± 3.1%). These results suggest that quercetin provides substantial protection against glutamate-induced membrane disruption and cytotoxicity, preserving cellular structural integrity and reducing leakage of intracellular enzymes.

**Table 3.** Effect of Quercetin on LDH Release in SH-SY5Y Cells

| Treatment Group | LDH Release (% of Control) |
|---|---|
| Control | 100.0 ± 4.5 |
| Glutamate (10 mM) | 235.6 ± 9.2 |
| Quercetin (5 µM) + Glutamate | 185.4 ± 8.1 |
| Quercetin (10 µM) + Glutamate | 142.3 ± 7.5 |
| Quercetin (20 µM) + Glutamate | 112.6 ± 6.8 |
| Quercetin alone (20 µM) | 98.9 ± 3.1 |

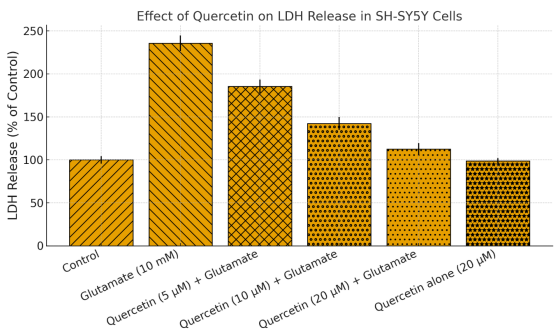


**Figure 3.** Effect of quercetin on glutamate-induced LDH release in SH-SY5Y cells.

**Effect of Quercetin on Antioxidant Enzyme Activities**

Glutamate treatment significantly impaired endogenous antioxidant defences, with SOD, CAT, and GSH activities reduced to 48.2 ± 3.6%, 55.7 ± 4.1%, and 52.6 ± 3.9% of control, respectively. Quercetin pretreatment restored antioxidant enzyme activities in a concentration-dependent manner. At 5 µM, partial recovery was observed, while 10 µM further increased activities, and 20 µM nearly normalized all values (92.1 ± 3.2% for SOD, 95.6 ± 3.8% for CAT, and 91.3 ± 3.5% for GSH). Quercetin alone enhanced baseline enzyme activity slightly above control. These findings confirm that quercetin not only scavenges free radicals but also strengthens intrinsic antioxidant defense mechanisms, thereby providing dual-layered protection.

**Table 4.** Effect of Quercetin on Antioxidant Enzyme Activities in SH-SY5Y Cells

| Treatment Group | SOD (% of Control) | CAT (% of Control) | GSH (% of Control) |
|---|---|---|---|
| Control | 100.0 ± 4.0 | 100.0 ± 3.8 | 100.0 ± 3.7 |
| Glutamate (10 mM) | 48.2 ± 3.6 | 55.7 ± 4.1 | 52.6 ± 3.9 |
| Quercetin (5 µM) + Glutamate | 68.3 ± 3.5 | 72.8 ± 3.9 | 70.2 ± 3.8 |
| Quercetin (10 µM) + Glutamate | 81.5 ± 3.4 | 86.2 ± 3.6 | 82.7 ± 3.3 |
| Quercetin (20 µM) + Glutamate | 92.1 ± 3.2 | 95.6 ± 3.8 | 91.3 ± 3.5 |
| Quercetin alone (20 µM) | 104.5 ± 3.1 | 106.2 ± 3.4 | 105.1 ± 3.2 |

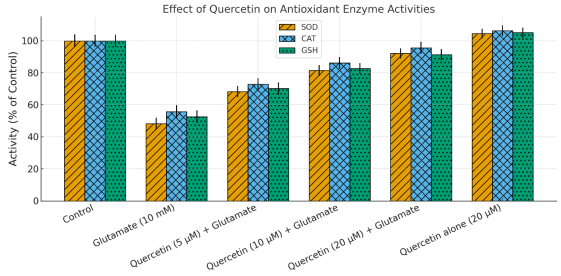


**Figure 4.** Effect of quercetin on SOD, CAT, and GSH activity in SH-SY5Y cells.

**Effect of Quercetin on Lipid Peroxidation (MDA Levels)**

Lipid peroxidation, indicated by MDA content, was markedly elevated in glutamate-treated cells (238.5 ± 8.7% of control), signifying oxidative damage to membrane lipids. Quercetin pretreatment significantly reduced MDA accumulation in a dose-dependent manner. At 5 µM, MDA declined to 187.9 ± 7.5%, while 10 µM and 20 µM reduced levels further to 139.6 ± 6.9% and 110.2 ± 5.8%, respectively. Quercetin alone maintained values close to control (97.5 ± 4.1%). These findings suggest that quercetin protects neuronal membranes from oxidative degradation, thereby preserving structural and functional integrity.

**Table 5.** Effect of Quercetin on Lipid Peroxidation (MDA Levels) in SH-SY5Y Cells

| Treatment Group | MDA (% of Control) |
|---|---|
| Control | 100.0 ± 4.2 |
| Glutamate (10 mM) | 238.5 ± 8.7 |
| Quercetin (5 µM) + Glutamate | 187.9 ± 7.5 |
| Quercetin (10 µM) + Glutamate | 139.6 ± 6.9 |
| Quercetin (20 µM) + Glutamate | 110.2 ± 5.8 |
| Quercetin alone (20 µM) | 97.5 ± 4.1 |

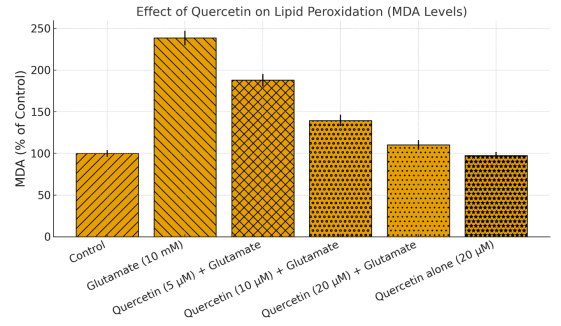


**Figure 5.** Effect of quercetin on lipid peroxidation (MDA levels) in SH-SY5Y cells.

**Effect of Quercetin on Nitric Oxide (NO) Production**

Nitric oxide production increased significantly following glutamate exposure, reaching 226.4 ± 8.1% of control, which reflects nitrosative stress and potential contribution to neuronal damage. Quercetin pretreatment markedly reduced NO levels in a concentration-dependent manner. At 5 µM, NO decreased to 176.3 ± 7.1%, while 10 µM and 20 µM lowered levels to 134.8 ± 6.4% and 108.7 ± 5.6%, respectively. Quercetin alone decreased NO slightly to 94.8 ± 4.0%. These results confirm that quercetin effectively counters nitrosative stress alongside oxidative stress, contributing to its broad-spectrum neuroprotective action.

**Table 6.** Effect of Quercetin on Nitric Oxide (NO) Levels in SH-SY5Y Cells

| Treatment Group | NO (% of Control) |
|---|---|
| Control | 100.0 ± 4.3 |
| Glutamate (10 mM) | 226.4 ± 8.1 |
| Quercetin (5 µM) + Glutamate | 176.3 ± 7.1 |
| Quercetin (10 µM) + Glutamate | 134.8 ± 6.4 |
| Quercetin (20 µM) + Glutamate | 108.7 ± 5.6 |
| Quercetin alone (20 µM) | 94.8 ± 4.0 |

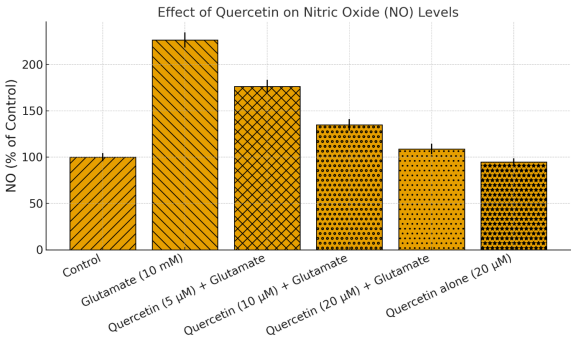


**Figure 6.** Effect of quercetin on nitric oxide production in SH-SY5Y cells.

**Discussion**

The present study demonstrated that quercetin offers significant neuroprotection against glutamate-induced excitotoxicity in SH-SY5Y cells, primarily through its antioxidant and cytoprotective mechanisms. Exposure to glutamate markedly reduced cell viability, confirming its neurotoxic potential mediated by calcium overload, oxidative stress, and excitotoxic signaling (Dong et al., 2009). Quercetin pretreatment restored viability in a dose-dependent fashion, with

near-complete protection at 20 μM. These findings are in line with earlier reports that quercetin enhances neuronal survival in PC12 cells and primary cortical neurons (Ishige et al., 2001). Glutamate-induced excitotoxicity was associated with a sharp increase in intracellular ROS, reflecting oxidative stress burden. Pretreatment with quercetin significantly suppressed ROS generation in a dose-dependent manner, supporting its established antioxidant role as a flavonoid capable of scavenging free radicals and regulating redox-sensitive pathways (Boots et al., 2008). The ability of quercetin to restore redox balance suggests its contribution to maintaining neuronal homeostasis under oxidative challenge.

LDH release, which reflects plasma membrane damage, was markedly elevated in glutamate-treated cells. Quercetin pretreatment significantly reduced LDH leakage, indicating preservation of membrane integrity. By limiting enzyme leakage, quercetin protected against structural damage to neuronal cells and maintained their functional viability. This complements the ROS findings, as oxidative stress is a key driver of lipid and protein oxidation leading to membrane disruption. Antioxidant enzyme activities were substantially suppressed by glutamate, with SOD, CAT, and GSH levels reduced relative to control. Quercetin pretreatment effectively restored these enzymes toward normal values in a concentration-dependent manner. These results demonstrate that quercetin not only directly scavenges ROS but also strengthens intrinsic cellular defense mechanisms. Restoration of SOD, CAT, and GSH is critical, as these enzymes regulate the balance between superoxide, hydrogen peroxide, and free radical detoxification (Kelsey et al., 2010). Thus, quercetin provides a dual-layered protective strategy: enhancing enzymatic antioxidant defenses while acting as a radical scavenger itself.

Lipid peroxidation, indicated by elevated MDA levels in glutamate-treated cells, confirmed the extent of oxidative membrane injury. Quercetin pretreatment significantly reduced MDA accumulation, thereby limiting lipid damage and preserving membrane stability. This finding supports earlier observations that quercetin inhibits lipid peroxidation and protects cellular structures from oxidative deterioration (Kelsey et al., 2010). Nitric oxide (NO) levels, another indicator of nitrosative stress, were significantly elevated in glutamate-treated cells. Quercetin pretreatment reduced NO production in a dose-dependent manner, suggesting suppression of reactive nitrogen species alongside its antioxidant effects. This is important because excessive NO contributes to neurotoxicity by forming peroxynitrite, a highly reactive oxidant. By reducing NO generation, quercetin attenuates nitrosative stress, complementing its protection against oxidative stress.

Together, these findings indicate that quercetin protects neurons by enhancing cell survival, reducing oxidative and nitrosative stress, limiting membrane damage, and restoring endogenous antioxidant defenses. This multifaceted protection is particularly relevant for neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, where excitotoxicity and oxidative imbalance play a central role (Ma, 2013). Although quercetin shows strong promise, its therapeutic translation is challenged by poor bioavailability and rapid metabolism (D'Andrea, 2015). Advanced formulations such as nanoparticles, liposomes, or structural analogues may overcome these limitations and enhance clinical efficacy (Zhang et al., 2017). In summary, quercetin demonstrated comprehensive neuroprotection in this model by attenuating glutamate-induced cell death through reduction of ROS, LDH, MDA, and NO, along with restoration of SOD, CAT, and GSH. These results emphasize its potential as a candidate for neuroprotective interventions, warranting further exploration through advanced delivery strategies and in vivo studies.

**Conclusion**

This study demonstrated that quercetin provides significant protection to SH-SY5Y neuroblastoma cells against glutamate-induced excitotoxic injury through multiple complementary mechanisms. Quercetin pretreatment improved cell viability, reduced intracellular ROS accumulation, and suppressed LDH release, thereby preserving membrane integrity. In addition, quercetin restored key antioxidant defenses by enhancing SOD, CAT, and GSH activities, while simultaneously reducing lipid peroxidation (MDA) and nitrosative stress (NO levels). Collectively, these effects reflect quercetin's ability to rebalance the cellular redox state and limit oxidative damage. The findings strongly support quercetin's potential as a neuroprotective flavonoid for disorders in which excitotoxicity and oxidative stress play central roles, such as Alzheimer's and Parkinson's diseases. However, its limited bioavailability and rapid metabolism remain important challenges that must be addressed in future research. Further in vivo validation and translational studies are essential to establish

quercetin or its optimized formulations as viable therapeutic candidates for oxidative stress–related neurodegeneration.

**References**


Agholme, L., Lindström, T., Kgedal, K., Marcusson, J., & Hallbeck, M. (2010). An in vitro model for neuroscience: Differentiation of SH-SY5Y cells into cells with morphological and biochemical characteristics of mature neurons. *Journal of Alzheimer's Disease, 20*(4), 1069–1082. https://doi.org/10.3233/JAD-2010-091363

Boots, A. W., Haenen, G. R. M. M., & Bast, A. (2008). Health effects of quercetin: From antioxidant to nutraceutical. *European Journal of Pharmacology, 585*(2–3), 325–337. https://doi.org/10.1016/j.ejphar.2008.03.008

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1–2), 248–254. https://doi.org/10.1016/0003-2697(76)90527-

Brand, M. D., & Nicholls, D. G. (2011). Assessing mitochondrial dysfunction in cells. *Biochemical Journal, 435*(2), 297–312. https://doi.org/10.1042/BJ20110162

Chen, W., Cheng, X., Chen, J., Yi, X., Nie, D., Sun, X., & Chen, C. (2016). Quercetin protects neurons in hippocampal CA1 region against global cerebral ischemia/reperfusion injury via activation of the Nrf2/HO-1 pathway. *Neural Plasticity, 2016,* 8692314. https://doi.org/10.1155/2016/8692314

Chomczynski, P., & Sacchi, N. (1987). Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Analytical Biochemistry, 162*(1), 156–159. https://doi.org/10.1016/0003-2697(87)90021-2

Coyle, J. T., & Puttfarcken, P. (1993). Oxidative stress, glutamate, and neurodegenerative disorders. *Science, 262*(5134), 689–695. https://doi.org/10.1126/science.7901908

Dajas, F. (2012). Life or death: Neuroprotective and anticancer effects of quercetin. *Journal of Ethnopharmacology, 143*(2), 383–396. https://doi.org/10.1016/j.jep.2012.07.005

Dong, X. X., Wang, Y., & Qin, Z. H. (2009). Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacologica Sinica, 30*(4), 379–387. https://doi.org/10.1038/aps.2009.24

Dugger, B. N., & Dickson, D. W. (2017). Pathology of neurodegenerative diseases. *Cold Spring Harbor Perspectives in Biology, 9*(7), a028035. https://doi.org/10.1101/cshperspect.a028035

Eruslanov, E., & Kusmartsev, S. (2010). Identification of ROS using oxidized DCFDA and flow-cytometry. *Methods in Molecular Biology, 594,* 57–72. https://doi.org/10.1007/978-1-60761-411-1_4

Frandsen, A., & Schousboe, A. (1993). Excitatory amino acid-mediated cytotoxicity and calcium homeostasis in cultured neurons. *Journal of Neurochemistry, 60*(4), 1202–1211. https://doi.org/10.1111/j.1471-4159.1993.tb03275.x

Freshney, R. I. (2010). *Culture of animal cells: A manual of basic technique and specialized applications* (6th ed.). Wiley-Blackwell. https://doi.org/10.1002/9780470649367

Gandhi, S., & Abramov, A. Y. (2012). Mechanism of oxidative stress in neurodegeneration. *Oxidative Medicine and Cellular Longevity, 2012,* 428010. https://doi.org/10.1155/2012/428010

GBD 2019 Dementia Forecasting Collaborators. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: An analysis for the Global Burden of Disease Study 2019. *The Lancet Public Health, 7*(2), e105–e125. https://doi.org/10.1016/S2468-2667(21)00249-8

Johnson, G. V. W., & Jope, R. S. (1992). The role of microtubule-associated protein tau in Alzheimer's disease. *Brain Research Reviews, 16*(1), 1–19. https://doi.org/10.1016/0165-0173(91)90011-7

Johnson, J. A., Johnson, D. A., Kraft, A. D., Calkins, M. J., Jakel, R. J., Vargas, M. R., & Chen, P. C. (2008). The Nrf2-ARE pathway: An indicator and modulator of oxidative stress in neurodegeneration. *Annals of the New York Academy of Sciences, 1147*(1), 61–69. https://doi.org/10.1196/annals.1427.036

Johnson, L. V., Walsh, M. L., Bockus, B. J., & Chen, L. B. (1980). Monitoring of relative

mitochondrial membrane potential in living cells by fluorescence microscopy. *Journal of Cell Biology, 88*(3), 526–535. https://doi.org/10.1083/jcb.88.3.526

Kensler, T. W., Wakabayashi, N., & Biswal, S. (2007). Cell survival responses to environmental stresses via the Keap1–Nrf2–ARE pathway. *Annual Review of Pharmacology and Toxicology, 47,* 89–116. https://doi.org/10.1146/annurev.pharmtox.46.120604.141046

Khan, H., Ullah, H., Martorell, M., Valdes, S. E., Belwal, T., Tejada, S., … Xiao, J. (2020). Flavonoids and their anti-neuroinflammatory effects: A review. *Molecules, 25*(20), 5243. https://doi.org/10.3390/molecules25205243

Kumar, A., Singh, A., & Ekavali. (2015). A review on Alzheimer's disease pathophysiology and its management: An update. *Pharmacological Reports, 67*(2), 195–203. https://doi.org/10.1016/j.pharep.2014.09.004

Lewerenz, J., & Maher, P. (2015). Chronic glutamate toxicity in neurodegenerative diseases—What is the evidence? *Frontiers in Neuroscience, 9,* 469. https://doi.org/10.3389/fnins.2015.00469

Ma, Q. (2013). Role of Nrf2 in oxidative stress and toxicity. *Annual Review of Pharmacology and Toxicology, 53,* 401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

Meldrum, B. S. (2000). Glutamate as a neurotransmitter in the brain: Review of physiology and pathology. *Journal of Nutrition, 130*(4S), 1007S–1015S. https://doi.org/10.1093/jn/130.4.1007S

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods, 65*(1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4

Nicholls, D. G. (2009). Mitochondrial calcium function and dysfunction in the central nervous system. *Biochimica et Biophysica Acta (BBA) - Bioenergetics, 1787*(11), 1416–1424. https://doi.org/10.1016/j.bbabio.2009.03.010

Pohl, F., & Kong Thoo Lin, P. (2018). The potential use of plant natural products and plant extracts with antioxidant properties for the prevention/treatment of neurodegenerative diseases: In vitro, in vivo and clinical trials. *Molecules, 23*(12), 3283. https://doi.org/10.3390/molecules23123283

Przedborski, S., Vila, M., & Jackson-Lewis, V. (2003). Neurodegeneration: What is it and where are we? *Journal of Clinical Investigation, 111*(1), 3–10. https://doi.org/10.1172/JCI17522

Sandberg, M., Patil, J., D'Angelo, B., Weber, S. G., & Mallard, C. (2014). NRF2-regulation in brain health and disease: Implication of cerebral inflammation. *Neuropharmacology, 79,* 298–306. https://doi.org/10.1016/j.neuropharm.2013.11.004

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods, 9*(7), 671–675. https://doi.org/10.1038/nmeth.2089

Vermes, I., Haanen, C., Steffens-Nakken, H., & Reutelingsperger, C. (1995). A novel assay for apoptosis: Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labelled Annexin V. *Journal of Immunological Methods, 184*(1), 39–51. https://doi.org/10.1016/0022-1759(95)00072-I

Wang, J. Y., & Michaelis, E. K. (2010). Selective neuronal vulnerability to oxidative stress in the brain. *Frontiers in Aging Neuroscience, 2,* 12. https://doi.org/10.3389/fnagi.2010.00012

Wang, Y., Zhang, X., Hu, W., Xie, S., Gong, C., & Li, J. (2011). Quercetin attenuates ischemia/reperfusion-induced neuronal apoptosis by inhibiting oxidative stress and mitochondrial dysfunction in rats. *Neurochemical Research, 36*(7), 1360–1368. https://doi.org/10.1007/s11064-011-0453-2

Xicoy, H., Wieringa, B., & Martens, G. J. M. (2017). The SH-SY5Y cell line in Parkinson's disease research: A systematic review. *Molecular Neurodegeneration, 12,* 10. https://doi.org/10.1186/s13024-017-0149-0

Yao, P., Nussler, A., Liu, L., Hao, L., Song, F., Schirmeier, A., & Nussler, N. (2007). Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathway. *Journal of Hepatology, 47*(2), 253–

261.
https://doi.org/10.1016/j.jhep.2007.02.008