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Expert Opinion Article

Unraveling the Role of Luteolin from *Avena Sativa* in Alzheimer's Disease: Targeting Key molecular mechanisms and its Neuroprotective Effects.

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ABSTRACT

Neurodegenerative diseases, marked by progressive neuronal dysfunction and loss, pose significant health challenges with glutamate accumulation contributing to neuronal cell death in conditions such as Alzheimer's disease. It is a progressive neurodegenerative disorder characterized by cognitive decline, memory loss and behavioral impairments that disrupt daily activities. It's the most common form of dementia, accounting for 60-70% of cases. Alzheimer's disease involves the degeneration of brain tissue, including loss of nerve cells and reduced responsiveness to neurotransmitters. Amyloid plaques and neurofibrillary tangles composed of hyperphosphorylated protein contribute to neuronal degeneration. This study explores the neuroprotective potential of Avena sativa leaf extract and its major constituent, Luteolin, against glutamate-induced hippocampal neuronal cell death. Treatment with glutamate led to reduced cell viability, altered morphology, increased reactive oxygen species (ROS) and apoptosis in HT-22 cells. However, pre-treatment with Avena sativa extract and luteolin attenuated these effects, restoring mitochondrial function, decreasing mitochondrial superoxide, and preserving mitochondrial morphology. Notably, luteolin inhibited excessive mitophagy by reducing lysosomal activity. Furthermore, luteolin's neuroprotective effects were associated with the activation of mTORC1, which attenuated glutamate-induced autophagy-mediated cell death. These findings highlight the potential of A.sativa and Luteolin as neuroprotective agents, regulating autophagy and mitochondrial dynamics to inhibit glutamate-induced neurotoxicity. This study provides valuable insights into the therapeutic potential of Luteolin and A. sativa in mitigating neurodegenerative diseases.

Key words: Neuroprotection, Luteolin, Avena sativa , Autophagy, Alzheimer's disease(AD)

INTRODUCTION

Neurodegenerative diseases are a group of conditions characterized by the progressive loss of neuronal structure and function in the central nervous system (CNS) and peripheral nervous system (PNS). This degeneration leads to various clinical features, including movement disorders, cognitive impairment, and behavioral changes[1,2].Recent research has identified eight hallmarks of neurodegenerative diseases, which provide a framework for understanding The accumulation of abnormal protein aggregates, such as amyloid-beta, alpha-synuclein, which contribute to neuronal damage and death. Neuroinflammation, including astrogliosis and microgliosis, plays a significant role in neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis[3]. The loss of neurons due to various mechanisms, including apoptosis, necrosis, autophagy and disruptions in synaptic function and neuronal networks, leading to impaired communication between neurons.

Alzheimer's disease (AD) is a progressive and debilitating condition that affects millions of people worldwide, particularly the elderly population. Characterized by severe cognitive decline, memory impairment, and behavioral disturbances, AD is pathologically defined by the accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated proteins[4-7]. These hallmark abnormalities disrupt synaptic communication, trigger neuroinflammation, and promote neuronal apoptosis, leading to

extensive neurodegeneration and cognitive dysfunction[8].

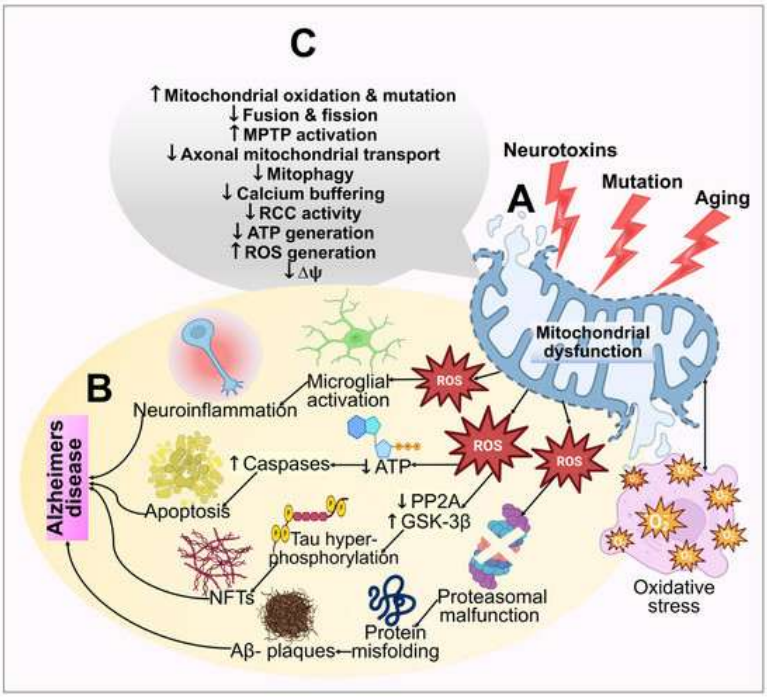

Fig 1 showed cellular and molecular mechanism underlying Alzheimer disease

Despite decades of research, the exact mechanisms driving Alzheimer's disease remains incompletely understood, highlighting the need for continued investigation into its pathophysiology and the development of effective therapeutic interventions[9,10]. The growing prevalence of Alzheimer's disease imposes a significant burden on patients, caregivers and healthcare systems, underscoring the urgent need for disease-modifying treatments that go beyond symptomatic relief. A comprehensive therapeutic approach targeting multiple pathological pathways simultaneously may be necessary to effectively treat Alzheimer's disease[11].

Recent advancements in neuropharmacology have intensified the search for multi-targeted therapeutic agents, particularly plant-derived compounds that exhibit neuroprotective, anti-inflammatory and antioxidant properties .These natural products have garnered increasing attention due to their ability to modulate multiple pathological features of AD, including oxidative stress, mitochondrial dysfunction, amyloid-beta aggregation and neuroinflammation[12]. The integration of systems biology and pharmacogenomic tools has further enabled researchers to incorporate these agents into personalized medicine frameworks, tailoring interventions to individual molecular and genetic profiles[13] .

Glutamate is the most abundant excitatory neurotransmitter in the central nervous system (CNS), which plays a crucial role in synaptic communication and neuronal signaling. In conditions such as Alzheimer's, there is an abnormal accumulation of glutamate in the brain's extracellular space. The glutamate accumulation is the primary factor responsible for excessive ROS generation via the overactivation of glutamate receptor N-methyl-D-aspartate (NMDA), resulting in an excess calcium influx to the cells[14-18]. Moreover, high extracellular glutamate can lead to glutathione depletion through cystine/glutamate antiporter. Subsequently, the accumulation of ROS leads to the deterioration of neuronal cells and triggers various types of cell death, such as apoptosis, necrosis and autophagy. Among those, autophagy is a cellular process involved in the degradation and recycling of cellular components, including damaged proteins and organelles[19,20]. In neurons, autophagy helps to remove misfolded proteins and damaged organelles, thus protecting neurons from cellular stress. However, under specific abnormal circumstances, such as prolonged nutrient dep rivation and chronic stress, autophagy can become excessive or uncontrolled, crossing a critical threshold where it triggers irreversible neuronal cell death. Moreover, autophagic cell death leads to the degradation of essential cellular components, especially mitochondria via mitophagy [21-23].The overactivation of the mitochondria degradation also process leads to an alteration in energy homeostasis. Consequently, inhibition of glutamate toxicity by targeting the excessive degradation process is regarded as a promising

strategy for alleviating neurodegenerative disease. The mammalian target of rapamycin complex 1(mTORC1) is a critical regulator of cell growth, metabolism, and autophagy. It plays a central role in coordinating cellular responses to various stress conditions, including nutrient deprivation, energy depletion, and other forms of cellular stress[24]. Under normal physiological conditions, mTORC1 fosters cell growth and protein synthesis. Simultaneously, it hampers autophagy by phosphorylating ULK1 (Unc-51-like autophagy activating kinase 1. However, mTORC1 activity is suppressed under stress conditions[25].

Luteolin a flavinoid derived from *Avena Sativum* leaves with antioxidant properties, has been shown to have beneficial effects in protecting against neurodegenerative diseases. Recent studies have demonstrated that Luteolin exhibits protective effects against neuronal damage caused by glutamate, a neurotransmitter that can induce excitotoxicity and neuronal apoptosis.

Luteolin have been shown to modulate key signaling pathways implicated in the pathogenesis of Alzheimer disease. It inhibits the MAPK pathway by reducing ERK1/2 phosphorylation, thereby attenuating oxidative stress and neuronal apoptosis[26]. Additionally, it suppresses NF-κB activation by preventing IκBα degradation and the nuclear translocation, resulting in reduced expression of pro-inflammatory cytokines. It exerts anti-inflammatory and neuroprotective effects by activating SIRT1, which in turn suppresses NF-κB signaling.

The study highlight strategies aimed to reduce amyloid accumulation, inhibiting phosphorylation, mitigating oxidative stress and promoting autophagy[27-28].By understanding the complex interplay between these pathological mechanisms, researchers can develop more effective treatments and improve patient outcomes.

METHODOLGY

1.Extract preparation

A.sativa leaves were dried in the shade for 5 days, ground and powdered. The extraction process involved placing leaf powders in a high-pressure cell and using liquid CO2 as the solvent, with ethanol added to enhance extraction efficiency. The effects of operating conditions, including temperature (5, 20, 25 °C), pressure (8.5, 10, and 14 MPa), and solvent composition (10 mole fraction of ethanol), on extraction yield were examined. The extraction time was set at 60 minutes, and the extracts were collected and analyzed using HPLC[29]. The study aimed to optimize the extraction conditions to achieve the highest yield of valuable compounds from A.sativum leaves, providing insights into the development of an efficient extraction method

2.Luteolin triggers the activation of mTORC1 to prevent glutamate-induced autophagy-mediated cell death.

The experiment involved extracting cells from mouse hippocampal tissue, specifically HT-22 cells, which were then used to study the effects of Luteolin on glutamate-induced autophagy-mediated cell death. The cells were pre-treated with Luteolin and then exposed to glutamate, after which cell viability was assessed using the MTT assay. This assay measures the reduction of MTT dye to formazan crystals by mitochondrial enzymes in viable cells, providing a quantitative measure of cell viability. The study aimed to investigate the potential neuroprotective effects of Luteolin by activating the mTORC1 pathway preventing glutamate-induced autophagy-mediated cell death. mTORC1 regulates autophagy and is often inhibited during stress conditions. In this study, HT-22 cells were pre-treated with Luteolin then exposed to glutamate and at last checked the viability of cells.

3.Cell Viability:

Cell viability was assessed using the MTT assay. The MTT assay is a widely used method for assessing cell viability and cytotoxicity. It is based on the reduction of the yellow MTT dye to purple formazan crystals by mitochondrial enzymes in viable cells. In this ,cells were incubated with MTT at a final concentration of 0.5 mg/mL for 4 hours. During this incubation period, viable cells with active mitochondria reduced the MTT dye to formazan crystals, which accumulated within the cells. The amount of formazan produced is directly proportional to the number of living cells. After the 4-hour incubation, DMSO (dimethyl sulfoxide) was added to dissolve the dark blue formazan crystals. DMSO is a solvent that effectively dissolves the formazan crystals, allowing for accurate measurement of the optical density.

The optical density (OD) of the dissolved formazan solution was is directly proportional to the number of viable cells[30]. A higher optical density value indicates a greater number of viable cells, while a lower value suggests reduced cell viability or cytotoxicity. By measuring the optical density,we determine the percentage of viable cells in a given sample

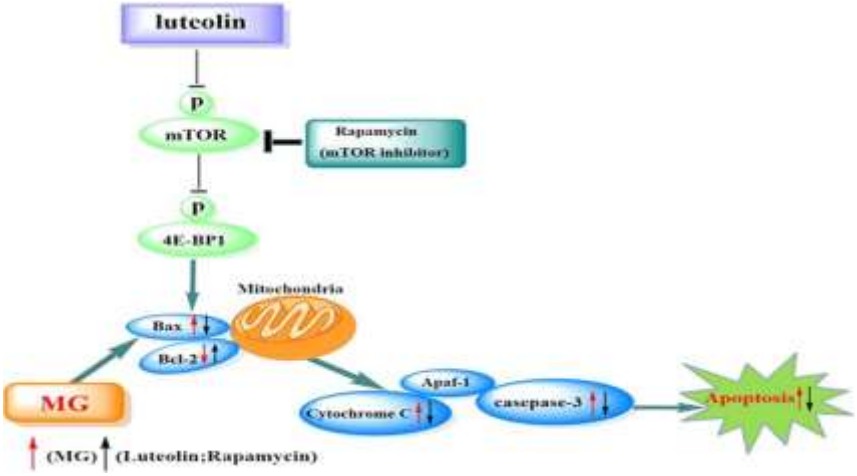

Fig 2 showed the Proposed Mechanism of Luteolin Against glutamate induced toxicity in Alzheimer disease

5. Mitochondrial Membrane Potential Assay

Cells were treated with glutamate and and then stained with JC-1 solution. Changes in mitochondrial membrane potential were measured using the JC-1 staining assay. Mitochondria were visualized using a fluorescence microscope, and the red/green fluorescence intensity ratio was used to assess mitochondrial depolarization.

RESULTS

Effect of Luteolin on cell viability in glutamate induced toxicity

To assess the impact of Luteolin on HT22 cells, cell viability was determined using the MTT assay after incubation with various concentrations of Luteolin (1.25, 2.5, 5, 10, 20 μM) for 24 h). The results indicated that Luteolin did not induce cytotoxicity in HT22 cells except at the highest concentration (20 μM) (Fig 3). Consequently, the maximum concentration of Luteolin was restricted to 10 μM in all subsequent experiments. Next, we evaluated the ability of Luteolin to counteract glutamate-induced cytotoxicity in HT22 cells. To determine the neuroprotective effect of Luteolin against glutamate-induced cytotoxicity in HT22 cells, the cells were treated with 20 mM glutamate with or without varying concentrations of Luteolin (1.25, 2.5, 5, and 10 μM) for 24 h. (Fig 2). Treatment with 20 mM glutamate significantly reduced the cell viability of HT22 neuronal cells compared to the control, which was reversed by pretreatment with 10 μM of Luteolin .

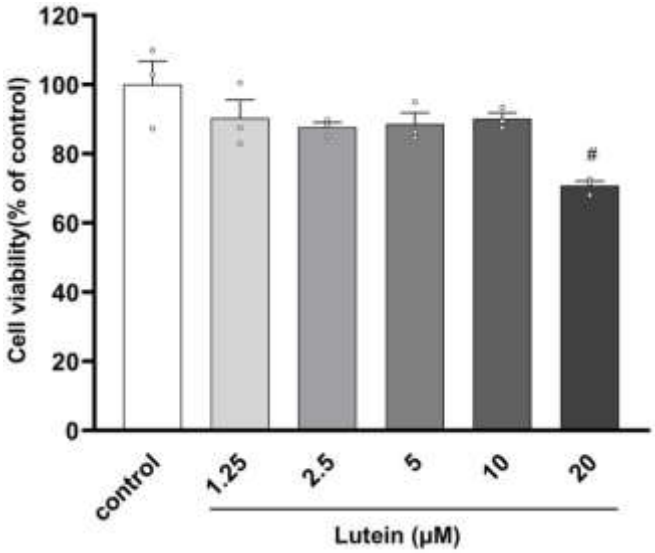

Fig 3 showed the Effect of Luteolin on the viability of HT22 cells. HT22 cells were incubated with various concentrations of Luteolin for 24 h, and cell viability was evaluated.

Consequently, it was observed that Luteolin significantly inhibited HT22 cell death induced by glutamate at a level similar to that of the positive control. Consistent with this findings, quantitative fluorescence intensity results demonstrated that glutamate exposure elevated increased intracellular ROS levels, a phenomenon significantly attenuated by pretreatment with 10 μM Luteolin These

findings suggested that the antioxidant properties of Luteolin may mitigate oxidative stress-mediated neuronal cell death induced by glutamate.

Previous studies have indicated that glutamate-induced cell death in HT22 cells primarily proceeds through the apoptotic pathway. In this study, we aimed to investigate the impact of luteolin on glutamate-induced apoptotic cell death. HT22 cells were treated with increasing concentrations of Luteolin in the presence of glutamate to assess its effects. DAPI staining demonstrated a reduction in glutamate-induced nuclear condensation following Luteolin treatment .Additionally, the number of positive HT22 cells induced by glutamate decreased significantly with increasing doses of Luteolin .Glutamate exposure led to elevated intracellular ROS levels, contributing to neuronal cell death through oxidative stress mechanisms To accomplish this, HT-22 cells were pre-treated with a range of luteolin concentrations (5–50 μM) for 24 h before exposing them to glutamate. The cell viability assay results demonstrated that luteolin effectively restores HT-22 cell viability .To explore Luteolin 's potential antiapoptotic mechanisms, its ability to counteract glutamate-induced apoptosis by preventing mitochondrial dysfunction triggered by oxidative stress.

Glutamate markedly induced mitochondrial membrane potential depolarization in HT22 cells However, pretreatment with Luteolin dose-dependently attenuated glutamate-induced MOMP depolarization . Additionally, immunoblot analysis revealed the activation of apoptosis due to glutamate-induced disruption of mitochondrial membranes .Conversely, Luteolin treatment dose-dependently inhibited apoptosis induction .These findings indicate that Luteolin exerts anti-apoptotic effects by inhibiting glutamate-induced mitochondrial apoptotic death in HT22 cells

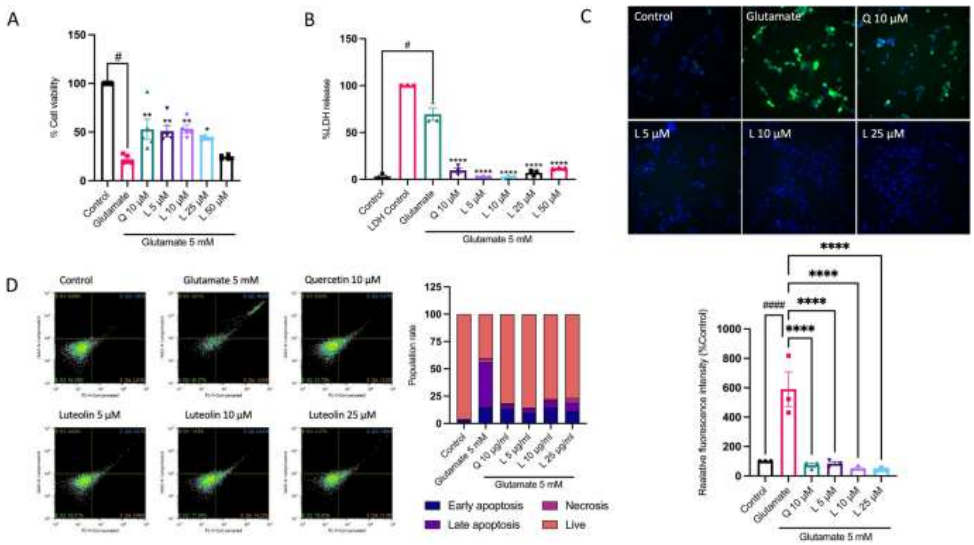

Fig 4 showed :A &B Luteolin increases the mTORC1 activation. HT-22 cells were pre-treated with luteolin at 5–25 μM for 24 h, followed by 5 mM glutamate for 18 h.C .The intracellular ROS was visualized under the CellInsight CX7 High-Content Screening (HCS) platform, the bottom bar graph shows the relative intracellular ROS level D.The HT-22 cells were stained with PE-Annexin V/7-AAD probes, the numbers of cell deaths were analyzed via flow cytometry

Luteolin exhibits antioxidant properties by regulating the signaling pathway in HT22 cells. It enhances Nrf2 translocation to the nucleus, leading to the activation of downstream target gene expression, including HO-1. This suggests Luteolin 's potential as a therapeutic agent for neurodegenerative disorders involving oxidative stress. By activating Nrf2 and inducing HO-1 expression. Luteolin may help mitigate oxidative stress and cell death, providing a potential neuroprotective effect. These findings demonstrate Luteolin 's antioxidant properties via regulation of the Nrf2/HO-1 axis, highlighting its potential as a neuroprotective agent. By activating Nrf2 and inducing HO-1 expression, Luteolin may help mitigate oxidative stress and cell death in neurodegenerative diseases. Luteolin mitigates glutamate-induced cytotoxicity in HT-22 and reduces cellular stress. Considering our results, luteolin has been selected as the primary compound for investigating its neuroprotective effect against glutamate-induced HT-22 hippocampal neuronal death.

DISCUSSION

Neuronal cell death is a complex process contributing to various neurological disorders and neurodegenerative diseases, with oxidative stress

playing a critical role. Excessive glutamate levels can trigger excitotoxicity, a cascade of events leading to neuronal cell death, and have been implicated in numerous neurodegenerative disorders[31]. This study investigates the protective effects of *A. Sativa* leaf extract and its primary active component, Luteolin, against glutamate-induced oxidative stress in HT-22 mouse hippocampal cells. These cells are particularly susceptible to glutamate toxicity due to the lack of N-methyl-D-aspartate (NMDA) receptors and exhibit Alzheimer's disease-specific markers under toxic conditions, making them a valuable model system for assessing potential anti-neurodegenerative disease agents[32-33]. By exploring the potential of ALE and luteolin to mitigate oxidative stress and neuronal cell death, this research aims to contribute to the development of novel therapeutic strategies for neurodegenerative diseases.

Our study demonstrates that ALE enhances the survival of neuronal cells against glutamate-induced neuronal cell death. ALE contains a substantial number of flavonoids, with quercetin and luteolin as major phytochemi cal components. Notably, quercetin is the predominant compound within ALE and has previously reported neuroprotective effects against glutamate oxidative toxicity in HT-22 cells[34]. On the other hand, luteolin is the second major phytochemical which is a natural flavonoid compound found in various fruits, egetables, and herbs. It is also reported in several studies that luteolin exhibits neuroprotective effects by acting as an antioxidant and anti-inflammatory agent[35].

Despite luteolin's established antioxidant and anti-inflammatory properties, its specific neuroprotective mechanisms against glutamate toxicity have remained unclear. Our findings demonstrate that luteolin effectively prevents glutamate-induced neuronal apoptosis and various forms of cell death, while also reducing the accumulation of intracellular reactive oxygen species (ROS)[36]. Notably, mitochondria play a crucial role in neuronal function and are involved in the intrinsic apoptotic pathway during glutamate excitotoxicity. Luteolin intervenes in this process, curbing mitochondrial dysfunction and restoring the count of functional mitochondria, likely due to its antioxidant activity. This is consistent with previous studies linking luteolin's antioxidant properties to reduced calcium levels and improved mitochondrial function. Mitochondrial dysfunction is a critical aspect of neuronal cell death , luteolin's ability to restore mitochondrial function and reduce oxidative stress suggests its potential as a neuroprotective compound. Previous in vivo studies have shown that luteolin ameliorates Alzheimer's disease symptoms by inhibiting endoplasmic reticulum stress ,reducing mitochondrial dysfunction and neuronal apoptosis[37-38]. Additionally, luteolin has been shown to mitigate hippocampal damage in stroke models by reducing glial cell activation and suppressing autophagy. While autophagy and mitophagy are essential processes for eliminating damaged cellular components, prolonged stress can provoke excessive autophagy and mitophagy responses, ultimately leading to neuronal cell death[39]. Luteolin's ability to regulate these processes may contribute to its neuroprotective effects, highlighting its potential as a therapeutic agent for neurodegenerative diseases characterized by mitochondrial dysfunction and oxidative stress.

The mechanistic target of rapamycin (mTOR) plays a pivotal role in regulating cellular processes, including cell growth, proliferation and autophagy, with significant implications for neuronal development, function and survival. Activation of mTORC1 can temper excessive autophagy, influence protein synthesis and remodel the cytoskeleton, thereby facilitating neuronal expansion. However, prolonged exposure to glutamate can disrupt mTOR signaling, leading to diminished phosphorylation of mTOR[40]. Notably, luteolin's activation of mTORC1 inhibits excessive autophagy and mitophagy triggered by glutamate which hinders the initiation of autophagy. Furthermore, luteolin's effects on autophagy and mitophagy are complex and context-dependent, involving the suppression of reactive oxygen species (ROS) accumulation, restoration of mitochondrial function, and activation of mTORC1. These findings suggest that luteolin may have therapeutic potential in neurodegenerative diseases characterized by mitochondrial dysfunction and oxidative stress.

CONFLICT OF INTEREST: Nil

CONCLUSION:

This study demonstrates the neuroprotective effects of luteolin, a compound found in A.Sativa leaves against glutamate-induced neuronal apoptosis. Luteolin effectively prevents neuronal cell death by reducing ROS accumulation, restoring mitochondrial function and mitigating mitochondrial dysfunction. Furthermore, luteolin curtails excessive autophagy and mitophagy, which are critical contributors to neuronal cell death. The antioxidant properties of luteolin are likely responsible for its protective effects. While these findings provide valuable insights into luteolin's potential as a therapeutic agent against neurodegenerative diseases, further investigations are necessary to fully understand its neuroprotective mechanisms and efficacy in animal and clinical studies. Ultimately, this research highlights the promise of luteolin as a potential treatment for neurodegenerative disorders, warranting further exploration and development.

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