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RESEARCH

Effects of quercetin-immobilized albumin cerium oxide nanoparticles on glutamate toxicity: in vitro study

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Abstract

One aspect of glutamate (Glut) toxicity may be the opening of the blood-brain barrier to albumin (Al), which in itself can cause nerve cell death. Quercetin (Q) is a polyphenolic substance and has a neuroprotective effect. Cerium oxide nanoparticles (Ce_2O_3NPs) are highly interested in biological applications due to their antioxidant properties. The current study aimed to investigate the impact of Q-immobilized Al+Ce_2O_3NPs in Glut-induced neurotoxicity, mainly focusing on cell viability and neurobiochemical changes. Hydrothermal synthesis and characterization of Q-immobilized Al+Ce_2O_3NPs were performed. After preparing the primary neuron culture, it was exposed to Glut to induce neurotoxicity. Then, various doses of Ce_2O_3NP, Al+Ce_2O_3NP, and Q+Al+Ce_2O_3NPs (1, 5, 10, and 25 µg/ml) were applied to the wells and incubated for 24 h. Then, cell viability was determined by MTT analysis. Additionally, oxidative stress parameters were measured. When the obtained data were examined, it was shown that cell viability decreased with Glut concentration but significantly increased with Q+Al+Ce_2O_3NPs treatment. When oxidative stress markers were considered, Glut treatment increased LDH, AChE, and TOS levels, while TAC and GSH levels decreased. However, the trend changed after Q+Al+Ce_2O_3NPs treatment, suggesting that damaged neurons were protected against oxidative stress. The results of this study indicate that Q+Al+Ce_2O_3NP can ameliorate Glut-induced neurotoxicity, especially when used at a dose of 25 µg/ml.

Keywords AcHE · Albumin · Ce_2O_3 nanoparticle · Glutamate · Neuron · Quercetin

Introduction

Excitotoxicity is defined as neuronal damage and death triggered by the toxic effects of excitatory neurotransmitters, mainly glutamate (Glut) (Dong et al. 2009). Therapies to prevent toxicity focus on blocking Glut receptors or limiting Glut release (Jia et al. 2015). However, these treatments interfere with physiological Glut functions and cause significant side effects (Sattler and Tymianski 2001). One aspect of Glut toxicity may be the opening of the blood-brain barrier to albumin, which in itself can cause nerve cell death. In vivo-vitro studies identified human serum albumin as the most likely neurotoxin in serum and showed that albumin has a potentiating effect on Glut-mediated neurotoxicity (Hooper et al. 2005; Nadal et al. 2001; Nadal et al. 1998; Nadal et al. 1996). Additionally, albumin can bind substances such as calcium ions and arachidonic acid, which play a role in Glut-mediated neurotoxicity (Nadal et al. 1996). Therefore, there is a need to develop alternative therapeutic strategies to restore normal brain function.

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One of the main challenges in neurological disorders is to develop an effective treatment method that can cross the blood-brain barrier (Saraiva et al. 2016). Nanoparticles are revolutionary for neurodegenerative diseases due to their targeted delivery and ability to overcome biological barriers (McNamara and Tofail 2017). Cerium oxide nanoparticles (Ce_2O_3NPs) are highly interested in biological applications due to their antioxidant properties. Since Ce_2O_3NPs can reversibly bind oxygen and switch between oxidation states (Caputo et al. 2014; Xu and Qu 2014), they can scavenge excess reactive oxygen species (ROS) in biological tissues (Heckert et al. 2008). It also exhibits antioxidative activities, including mimetic properties of superoxide dismutase (SOD) and catalase enzymes to scavenge nitric species (Heckert et al. 2008; Hosseini and Mozafari 2020). Unlike traditional antioxidants, the radical scavenging property of Ce_2O_3NPs is regenerative and allows activity over long periods (Rzigalinski et al. 2017). Previous studies have shown that Ce_2O_3NPs ameliorate neurodegeneration (Elshony et al. 2021) in a Parkinson's disease model, oxidative brain damage (Elshony et al. 2021), hepatic steatosis (Wasef et al. 2021), reproductive toxicity (Saleh et al. 2020), and drug-induced keratinocyte cytotoxicity (Singh et al. 2016).

Quercetin is a polyphenolic substance that can be easily found in diverse berries and vegetables (Havsteen 1983). It has neuroprotective effects in diverse neurodegenerative illnesses, including traumatic brain harm and paralysis. It also maintains against toxicity by alleviating the rise in calcium concentration in neuronal harm caused by Glut toxicity (Du et al. 2018; Park et al. 2020). However, it showed neuroprotective effects by reducing free radical species and controlling calcium concentrations in styrene oxide–induced neuronal cell demise (Sakanashi et al. 2008). It has also been reported to show neuroprotective effects by regulating the calcium sensor protein hypocalcin in an animal model of stroke (Park et al. 2020). Therefore, the present study aimed to investigate the effect of quercetin-immobilized albumin Ce_2O_3NPs in Glut-induced neurotoxicity, mainly focusing on cell viability and neurobiochemical changes.

Materials and methods

Hydrothermal synthesis and characterization of quercetin-immobilized albumin cerium oxide NPs

Ce2O3NPs were first synthesized using the hydrothermal method (Cicek et al. 2015). After the serum albumin solution prepared at a concentration of 1 mg/ml was added to the Ce2O3NPs medium, it was homogenized with high-frequency ultrasonic waves using an ultrasonic cell disperser. Then, after removing unbound serum albumin by washing with pure water, the solid material was treated with quercetin at a concentration of 1 mg/ml and homogenized with ultrasonic waves. After the immobilization process was completed, quercetin and immobilized albumin Ce2O3NPs, which were lyophilized in a vacuum environment, were stored at +4 °C until use.

The surface topography of Ce_2O_3NPs was characterized using a scanning electron microscope (SEM), specifically a Metek Apollo Prime with an active area of 10 mm^2 and a Microscope Inspect S50. For crystallinity analysis, X-ray diffraction (XRD) was employed using a Panalytic Empyrean system, which is equipped with a Ni-filtered CuKα radiation source ($\lambda = 0.1542$ nm). The XRD measurements were conducted over a 2θ range of 10 to 90° at a scanning rate of 4° per min. Fourier-transform infrared spectroscopy (FTIR) analysis of Ce_2O_3NPs was recorded using Vertex 80 Model FTIR Frontier spectrophotometer with attenuated total reflection technique in the 400–4000 cm^{-1} region

Primary neuron culture

This study was conducted with the approval of the Atatürk University Animal Experiments Local Ethics Committee (approval number E-42190979-000-2300239665). Newborn Sprague Dawley rat puppies, less than 24 h old, were used to obtain cortex neurons. After the pups were quickly decapitated, the extracted cortices were transferred to 5-ml Hanks' Balanced Salt solution, macro-disintegration was performed with the help of a scalpel, and then micro-disintegration was performed with Trypsin-Ethylenediaminetetraacetic acid (0.25% trypsin-0.02% EDTA) (Sigma, USA). After the digestion was completed, it was centrifuged at 1200 rpm for 5 min. Cellular medium (88% neuro basal medium, 10% fetal cattle solution, 2% B27, 0.1% antibiotics (amphotericin B, streptomycin, and penicillin,) was added to the cells that settled to the bottom. The cells were incubated for 10 days (5% CO_2 and 37 °C) by changing the medium every 3 days (Yeni et al. 2023).

To induce neurotoxicity, neurons other than the control group were exposed to Glut (Sigma, USA) at a concentration of 10^{-5} M for 5 min (Okkay et al. 2022). Then, to evaluate the role of Ce_2O_3 NPs, albumin (Al)+Ce_2O_3NPs, and quercetin (Q)+Al+Ce_2O_3NPs in Glut-induced neurotoxicity, various doses (1, 5, 10, and 25 µg/ml) were applied to separate wells and incubated for 24 h (Liu et al. (2024).

MTT (3-(4,5-dimetiltiazol-2-yl)-2,5-difeniltatrazilyum bromid) analysis

To determine the cytotoxicity of the materials, an evaluation was made with the MTT substance. The mixture prepared as 5-mg MTT powder (Sigma, USA) in 1 ml phosphate-buffered saline was passed through a 0.20-µm sterile filter, and

the outer surface was covered with aluminum foil and kept at +4 °C until it was used. After the media fluids of the incubated cells were withdrawn, the previously prepared samples were placed in each well and incubated again for 24 h at 37 °C in an environment containing 5% CO_2. To solubilize the formazan crystals formed after 24 h, dimethylsulfoxide was added at 100 µl/well and incubated again for 4 h. After this process, the absorbance was measured at a wavelength of 570 nm in a spectrophotometer (Yeni et al. 2024).

Markers of oxidative stress

To investigate oxidative damage, total antioxidant capacity (TAC), total oxidant status (TOS) (Rel Assay Diagnostics, Gaziantep, Turkey), lactate dehydrogenase (LDH), glutathione (GSH), and acetylcholinesterase (AChE) were tested using commercial kits in the supernatant samples obtained (Elabscience, USA). It was examined by the ELISA method.

Statistical analysis

Results are given as mean ± standard error. Statistical comparison between groups was calculated using one-way ANOVA and Tukey HSD method. SPSS 22.0 program was used for statistical analysis, and $p < 0.05$ was considered a statistically meaningful difference in all tests.

Results

SEM analysis of Ce_2O_3NPs

To determine the chemical and mineralogical compositions of the hydrothermal synthesized Ce_2O_3NPs, SEM analysis was employed. The SEM images of the Ce_2O_3NPs were captured at a magnification of × 5000 using a Metek Apollo Prime microscope with an active area of 10 mm^2, Microscope Inspect S50, and SE Detector R580. The results obtained are presented in Fig. 1.

From the observations in Fig. 1, it is evident that the majority of the Ce_2O_3NPs exhibit a spherical morphology. Close examination of the Ce_2O_3NPs in Fig. 1 reveals that they predominantly assume spherical shapes, with sizes ranging approximately from 10 to 30 nm (Sankar and Kumar 2021). The hydrothermal synthesis approach employed in this study aligns with the growing trend toward sustainable and environmentally friendly nanoparticle production methods. Hydrothermal synthesis not only reduces the use of hazardous chemicals but also utilizes natural resources, making it a more eco-friendly option (Nosrati et al. 2023).

XRD analysis of Ce_2O_3NPs

The XRD analysis of Ce_2O_3NPs was performed using a Rigaku D-Max 2000 XRD diffractometer (Rigaku, Tokyo, Japan). The measurements utilized CuKα radiation (λ =

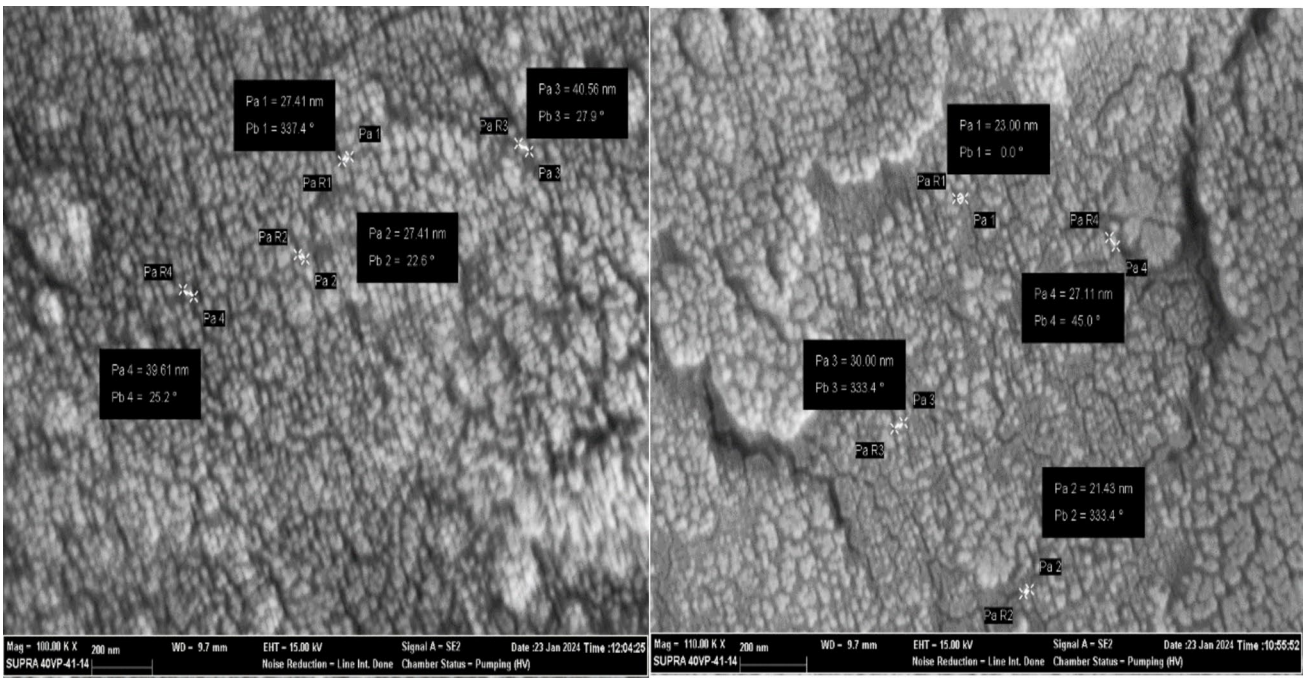

Fig. 1 SEM image of Ce_2O_3NPs

0.154 nm) over a 2θ range from 5 to 90°, with a step size of 0.1°. According to the Joint Committee on Powder Diffraction Standards (JCPDS) file number 65-5923, all observed diffraction peaks were attributed to Ce_2O_3NPs. The XRD pattern displayed five prominent peaks at 2θ angles of 30.96°, 34.49°, 35.6°, 50.37°, and 61.18° (Fig. 2). These peaks serve as characteristic fingerprints confirming the presence of Ce_2O_3NPs.

The XRD analysis provides critical insights into the structural properties of Ce_2O_3NPs. The observed diffraction peaks at specific 2θ angles align well with the standard diffraction data for Ce_2O_3, as referenced from the JCPDS file 65-5923. This alignment confirms the successful synthesis of Ce_2O_3NPs and validates their crystalline structure. The precise identification of these peaks is crucial, as it allows for the differentiation of Ce_2O_3 from other possible cerium oxide phases, such as CeO_2. The distinct peaks at 30.96°, 34.49°, 35.6°, 50.37°, and 61.18° serve as reliable indicators of the Ce_2O_3 phase, underscoring the material's purity and structural integrity (Nadaroglu et al. 2017).

Furthermore, the use of CuKα radiation with a wavelength of 0.154 nm provides high-resolution diffraction patterns, which are essential for accurate phase identification. The wide scanning range (5–90°) and small step size (0.1°) enhance the resolution and accuracy of the measurement, ensuring that even minor peaks are detected and correctly assigned. The structural information obtained from XRD analysis is pivotal for understanding the material properties of Ce_2O_3NPs. These properties include crystallite size, phase composition, and potential defects or strain within the crystal lattice.

FTIR analysis

To determine the functional groups, the FTIR spectrum was taken between 400 and 4000 cm^{-1} and is given in Fig. 3. The

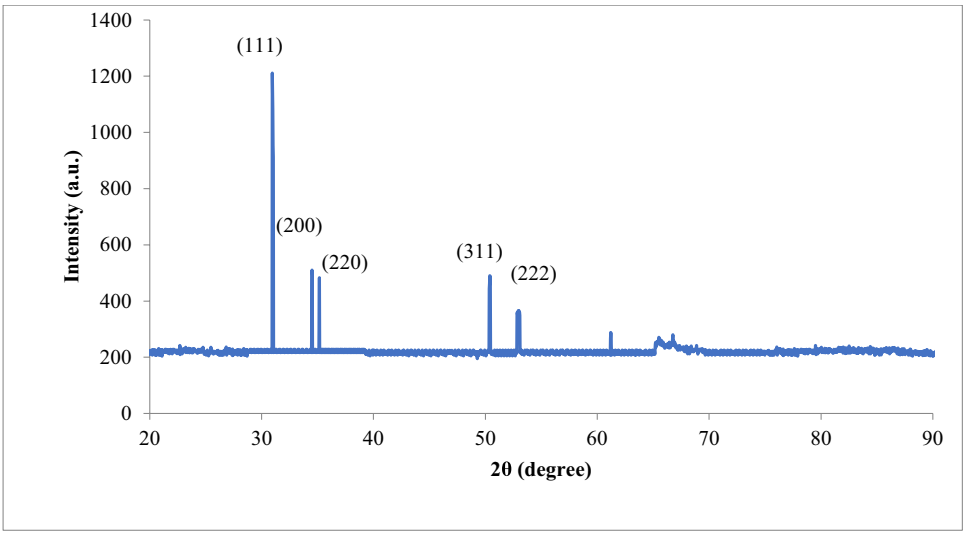

Fig. 2 XRD pattern of Ce_2O_3NPs

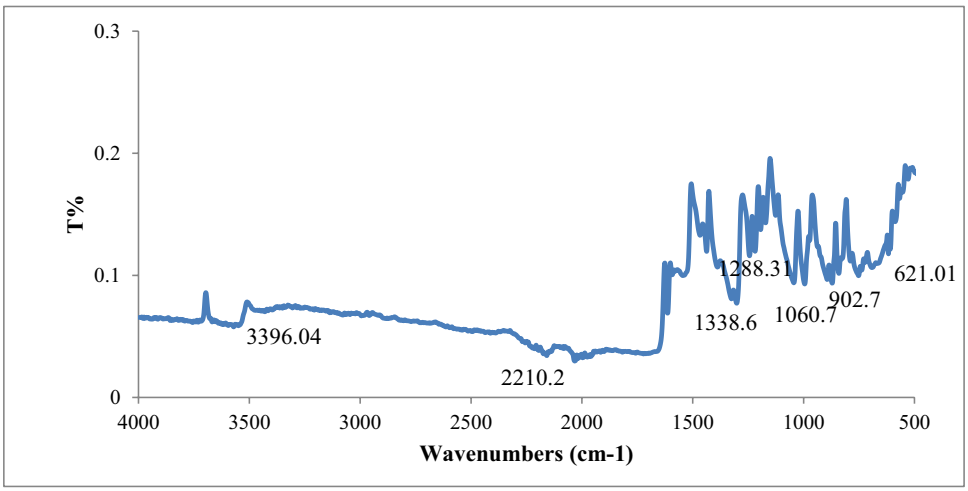

Fig. 3 FTIR spectra of the Ce_2O_3NPs

FTIR spectrum for Ce_2O_3NPs (Fig. 3) shows the following peaks: 3396 (br), 2210 (m), 1338 (w), 1288 m, 1060 (m), 902 (m), and 621 (w) cm^{-1}. These peaks indicate the presence of O−H, −C−C−, C=O, C−H, >C=C<, C−H, and −Ce=O bonds, respectively. In particular, the peak observed at 613 cm^{-1} indicates the formation of the metal-oxide bond (−Ce=O), which indicates the configuration of Ce_2O_3.

The porous structure on the surface structure of Ce_2O_3NPs has an important role in interactions with neuronal cells. When the SEM analysis of the Ce_2O_3 structure is examined, the homogeneous distribution of the observed morphological structures and the crystal structure determined in the XRD analysis reveal that Ce_2O_3NPs can be effective in cellular uptake. In addition, when the FTIR results of Ce_2O_3NPs are examined, it has been shown that there are hydroxyl groups on the surface of the nanoparticles. Thanks to these –OH groups, quercetin molecules are immobilized on the nanoparticles and their interactions with neuronal cells are easily carried out. Ce_2O_3NPs coated with quercetin can play a protective role in cell health through neuroprotective mechanisms by contributing to reducing oxidative stress and regulating cellular signaling pathways. These findings reveal how nanoparticles behave in the biological environment and the mechanism of action on cellular processes.

Effect of Q+Al+Ce_2O_3NPs on Glut-induced cell death

The cytotoxic effect of Ce_2O_3NP, Al+Ce_2O_3NP, and Q+Al+Ce_2O_3NPs was determined using the MTT method. The cytotoxic effect of the treatment agents applied at different concentrations (1, 5, 10, 25 µg/ml) on Glut-induced neurotoxicity created in primary neuron culture is shown in Fig. 4. At the end of 24 hours of incubation, it was observed that the cell viability rate of 10^{-5} M Glut decreased to 54.98% compared to the control group ($p < 0.001$). An increase in cell viability was detected following Ce_2O_3NP, Al+Ce_2O_3NP, and Q+Al+Ce_2O_3NP applications. The most significant increase was observed following the application of 10–25 µg/ml Q+Al+Ce_2O_3NP (82.94% and 97.95%, respectively), while the least increase was observed following 1 µg/ml Al+Ce_2O_3NP and Q+Al+Ce_2O_3NP (68.44% and 66.34%, respectively). It was observed that the groups applied with Ce_2O_3NP at a concentration of 25 µg/ml had a statistically significant protective effect on cell viability compared to Glut ($p < 0.05$), while the most significant protective effect was detected in the group applied with 25 µg/ml Q+Al+Ce_2O_3NP ($p < 0.001$).

In an in vitro osteoarthritis model study, it was reported that CeO_2NP-loaded hyaluronic acid inhibited H_2O_2-induced oxidative stress on chondrocytes (Lin et al. 2020). We confirmed the neuroprotective effect of Q+Al+Ce_2O_3NP on Glut-induced neurotoxicity. The protective effects of quercetin-immobilized Ce_2O_3NPs were dose-dependent. Therefore, we believe that Ce_2O_3 can protect quercetin from degradation and increase cell viability with its synergistic effect.

Markers of oxidative stress

LDH is a marker of cell death released into the culture medium from damaged cells. To test the neuroprotective potential of Ce_2O_3NP, Al+Ce_2O_3NP, and Q+Al+Ce_2O_3NPs, different nanoparticle concentrations (1, 5, 10, and 25 µg/ml) were applied to neuronal cells for 24 h. After incubation of neuronal cells with Glut (10^{-5} M), we observed an almost threefold increase in LDH release compared to the control group (295.39%; $p < 0.001$); this was significantly reduced (136.29%, 122.38%, 112.69%, and 102.53%; $p < 0.05$, $p < 0.001$, respectively) by all tested (1, 5, 10, and 25 µg/ml) Q+Al+Ce_2O_3NP concentrations (Fig. 5). In a recently published article, it was reported that polyacrylic

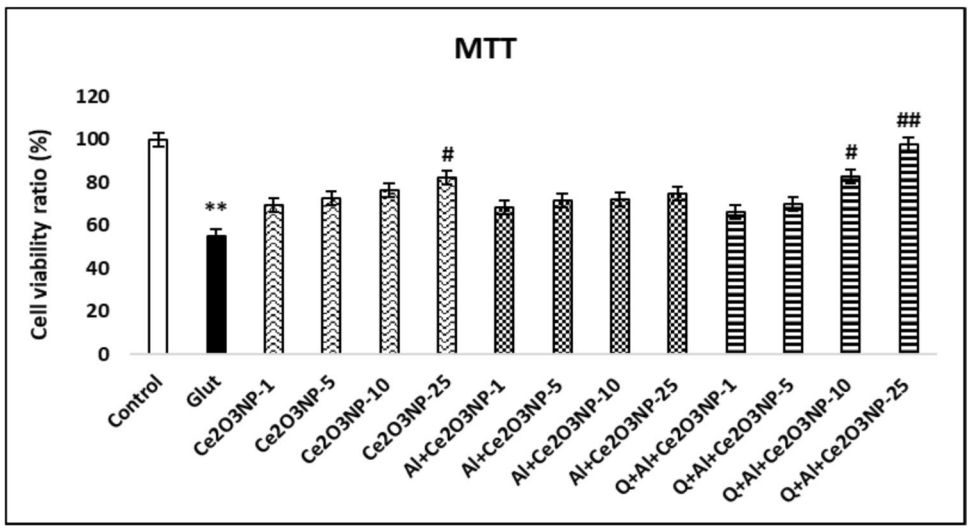

Fig. 4 Effects of Glut, Ce_2O_3NPs, AI+Ce_2O_3NPs, and Q+AI+Ce_2O_3NPs doses on cell viability in primary neuron culture. Cell viability was assessed with the MTT assay. (**Glut group showed a statistical difference ($p < 0.001$) compared to the control group. #Tagged groups showed a statistical difference ($p < 0.05$) compared to the Glut group. ##Tagged groups showed a statistical difference ($p < 0.001$) compared to the Glut group)

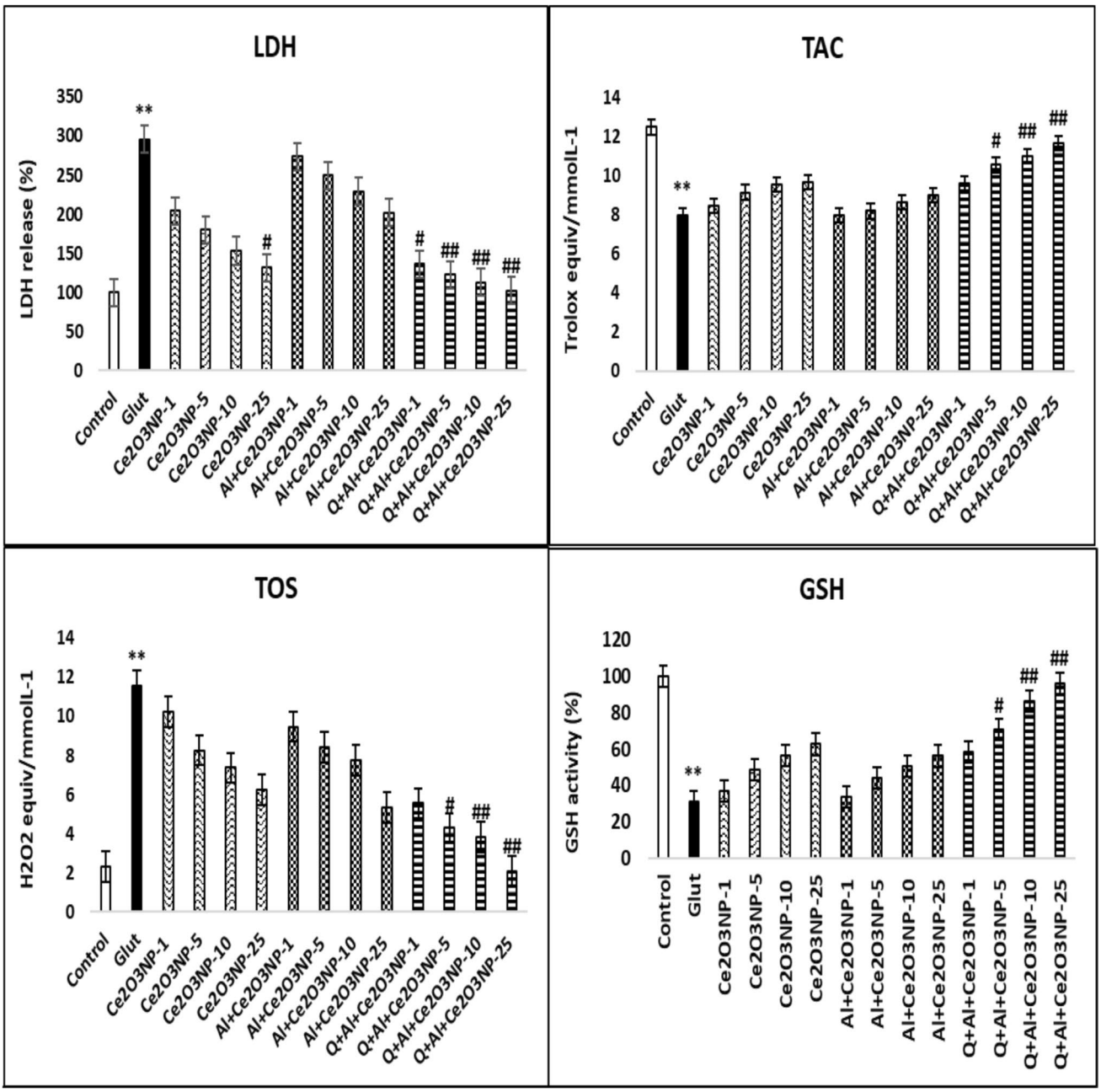

Fig. 5 Effects of Glut, Ce_2O_3NPs, AI+Ce_2O_3NPs, and Q+AI+Ce_2O_3NPs doses on antioxidant enzyme activities in primary neuron culture. LDH, TAC, GSH, and TOS activities were determined by ELISA kits. (**Glut group showed a statistical difference ($p < 0.001$) compared to the control group. #Tagged groups showed a statistical difference ($p < 0.05$) compared to the Glut group. ##Tagged groups showed a statistical difference ($p < 0.001$) compared to the Glut group)

acid conjugated CeONPs reduced neuronal cell death against H_2O_2 and 6-OHDA-induced cell damage in human neuroblastoma SH-SY5Y cells (Meenambal et al. 2023). In line with these data, our findings revealed the neuroprotective effect of Q+Al+Ce_2O_3NPs.

After the application of different concentrations of treatment agents (1, 5, 10, 25 µg/ml) for 24 h, the cell culture medium was taken. The antioxidant and oxidant capacities of the culture medium were measured with the help of a commercial kit. Glut TAC levels significantly reduced compared to the control group (7.96 mmol Trolox Equiv/L; $p < 0.001$). Treatment groups showed increased antioxidant capacity compared to the Glut group. In the groups applied with high doses 5, 10, and 25 µg/ml Q+Al+Ce_2O_3NP (10.56, 11.03, and 11.7 Trolox Equiv mmol/l−1 respectively; $p < 0.05$, $p < 0.001$), a significant increase in antioxidant capacity was

detected in parallel with the increase in dose compared to the Glut group. It was also found that the TAC results were parallel to the MTT results, and 25 µg/ml Q+Al+Ce_2O_3NP had the highest effect on both cell viability and antioxidant capacity (Fig. 5).

GSH is an important endogenous component of cellular antioxidant defense. Regarding GSH levels, Glut significantly reduced GSH activity (31.01%, $p < 0.001$) compared to control. On the other hand, dose-dependent Q+Al+Ce_2O_3NPs (5, 10, 25 µg/ml) groups showed higher GSH activities than the Glut group (70.37%, 86.34%, and 95.83%, respectively; $p < 0.05$, $p < 0.001$) which was observed to increase significantly. In addition, the GSH level in the high-dose Q+Al+Ce_2O_3NP group approached the control group (Fig. 5).

TOS results show the oxidant and free radical levels in the cell culture medium. The findings showed that Glut application significantly raised TOS levels compared to the control group (11.57 H_2O_2 mmol Equiv/l−1; $p < 0.001$). It also showed that 1 µg/ml Ce_2O_3NP and Al+Ce_2O_3NP applied groups had the highest oxidant capacity compared to the Glut group, which also showed intracellular stress factor-induced toxicity and increased cell death. In line with the TAC results, a statistically significant decrease in TOS levels was detected in the 5, 10, and 25 µg/ml Q+Al+Ce_2O_3NP applied groups compared to the Glut group (4.28, 3.83, and 2.10 H_2O_2 mmol Equiv/l−1, respectively; $p < 0.05$, $p < 0.001$). However, consistent with both our MTT and TAC results, the oxidant level was found to be the lowest following the application of 25 µg/ml Q+Al+Ce_2O_3NP (Fig. 5).

Nowadays, many drug delivery systems are being developed to increase the stability of quercetin. In the in vitro and in vivo study where CeO_2NPs were used to increase bioavailability, it was reported that it can inhibit neurotoxicity by enhancing antioxidant activity, protect neurons, and significantly improve cognitive deficits (Hu et al. 2023). Ersoz et al. used poly(lactic-co-glycolic acid) nanoparticles to load quercetin, which increased its cytotoxicity and antioxidant activity against C6 glioma cells (Ersoz et al. 2020). In this context, the data in our findings show the strong antioxidant capacity of Q+Al+Ce_2O_3NPs.

AChE inhibition

AChE is an enzyme responsible for the breakdown of ACh. AChE inhibition improves cognitive impairment by increasing ACh levels (Abdalla et al. 2014). In our study, Glut application significantly increased AChE activity compared to the control group (200.35%; $p < 0.001$) (Fig. 6). However, a significant decrease in AChE activities was observed in the dose-dependent Q+Al+Ce_2O_3NP (5, 10, 25 µg/ml) groups (90.73%, 80.42%, and 50.95%, respectively; $p < 0.05$, $p < 0.001$) compared to the Glut group. In vitro model studies have shown that quercetin administration leads to AChE enzyme inhibition, thus preventing the degradation of ACh (Khan et al. 2020). In another in vivo study model, it was reported that quercetin administration increased ACh levels in the synaptic cleft by inhibiting AChE in cadmium-exposed rats. This suggests that quercetin reduces the hydrolysis of ACh due to inhibition resulting from hydrophobic interactions and strong hydrogen bonds with the enzyme summarized as follows (Abdalla et al. 2014). Our findings are consistent with previous studies and show that Q+Al+Ce_2O_3NPs are effective in inhibiting AChE.

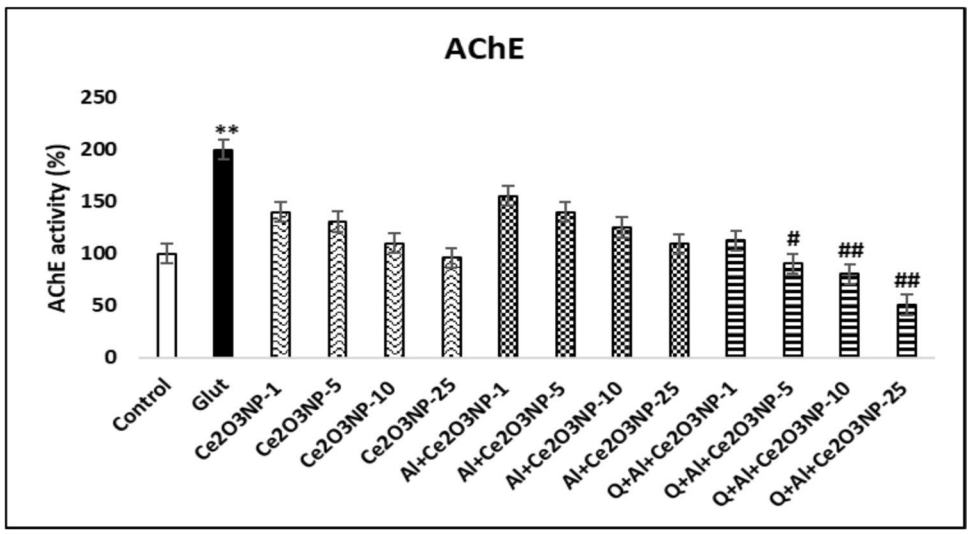

Fig. 6 Effects of Glut, Ce_2O_3NPs, AI+Ce_2O_3NPs, and Q+AI+Ce_2O_3NPs doses on AChE enzyme activities in primary neuron culture. AChE activity was determined by the ELISA kit. (**Glut group showed a statistical difference ($p < 0.001$) compared to the control group. #Tagged groups showed a statistical difference ($p < 0.05$) compared to the Glut group. ##Tagged groups showed a statistical difference ($p < 0.001$) compared to the Glut group)

Discussion

Glut, in addition to being the most significant excitatory neurotransmitter, is a strong neurotoxin. It is considered the primary reason for neuron demise in neurodegenerative illness. In Glut toxicity, the production of reactive oxygen species (ROS) increases, causing neuronal cell demise, and eventually, cell viability decreases (Duan et al. 2007; Ekinci et al. 2000). Quercetin protects HT22 cells from Glut-induced cell death by inhibiting the excessive production of intracellular ROS (Yang et al. 2013). Additionally, it is a strong neuroprotectant against oxidative neuronal damage in cortical cells (Dok-Go et al. 2003). MTT has become a very popular method for determining cell viability in cell culture studies. A recent in vitro study demonstrated that quercetin ameliorates neuronal cell death due to Glut toxicity by controlling the calcium-binding protein parvalbumin (Kang et al. 2022). In our study, a significant decrease in cell viability was detected in neuronal cells exposed to Glut. An increase in cell viability was detected following the application of therapeutic agents. This increase was observed to reach up to 82–97% at high doses. We confirmed that Q+Al+Ce2O3NPs repaired nerve damage caused by Glut-induced neurotoxicity and showed neuroprotective effects in a dose-dependent manner.

Recently, several studies have focused on the therapeutic potential of CeONPs in neurological disorders due to their potential antioxidant and antiapoptotic properties (Bailey et al. 2016; Heckman et al. 2013; Sandhir et al. 2015). Thanks to their potent and regenerative antioxidant effects, CeO_2NPs extended the lifespan of spinal and cortical neurons in cell culture, improved cell function, reduced oxidative stress–related apoptosis, increased dopamine secretion, and increased the transcription of genes associated with neuroprotection (Ciofani et al. 2013; Ciofani et al. 2014; Dowding et al. 2014). In support of this explanation, the present study showed a dose-dependent decrease in LDH and TOS, as well as an increase in GSH and TAC levels in neurons treated with the Q+Al+Ce_2O_3NP dose. Increased ROS production and decreased levels of the antioxidant compound GSH, antioxidant enzymes SOD, and GSH-Px were reported in neuronal cultures subjected to Glut toxicity (Shimmyo et al. 2008; Yu et al. 2005). Consistent with previous data, in this study, we found significantly elevated LDH and TOS levels and decreased GSH and TAC levels in neurons exposed to Glut. Numerous studies have reported that altered AChE levels cause neuronal degeneration in different stages of Alzheimer's disease (Darvesh 2016). Our findings show that Glut increases AChE activity, while Q+Al+Ce2O3NPs significantly decrease AChE activity. This indicates that Q+Al+Ce_2O_3NPs have a neuromodulatory effect.

Increased intracerebral Al levels are associated with Alzheimer's illness, multiple sclerosis, and paralysis pathology where blood-brain barrier harm is evident. The effects of Al on cells in the brain have been researched mainly in astrocytes. Since Al can activate microglia, which may exacerbate the neurodegenerative paths, the study in which they identified signaling cascades activated in microglia exposed to pure Al reported that therapy of primary cultured microglia and the N9 microglial cell line with pure Al caused an increase in intracellular calcium (Hooper et al. 2005). These data show that Al may play a role in microglial activation in pathological conditions. Similarly, in our study, it was found that Glut-induced neuron cells decreased in cell viability and antioxidant levels in the presence of Al compared to healthy cells. However, the opposite was observed in Al+Ce_2O_3NP treatment immobilized with quercetin. In this regard, Q+Al+Ce_2O_3NP showed neuroprotective effects by reducing ROS caused by both Glut and Al and controlling calcium concentrations.

CeONPs, which are widely applied today, have been recommended for the treatment of chronic diseases associated with oxidative stress due to their strong antioxidant properties (Rzigalinski et al. 2017; Cordoba-Jover et al. 2019; Tatar et al. 2018). It mimics natural antioxidant enzymes such as SOD and catalase, making CeONPs a profitable catalytic nanoparticle. It can neutralize excess ROS, which is associated with numerous pathologies (Turin-Moleavin et al. 2019). It has been shown to have protective effects in Parkinson's illness due to its ability to cross the blood-brain barrier, and this has been connected to the decrease of α-Synuclein fibrillation (Mohammad-Beigi et al. 2019). The results of one study demonstrated the synergistic effects of NPs in combination with antioxidants and the prevention of nanoparticle toxicity on cells (Ghafoori et al. 2017). However, with the survival of cerebral tissue cells greatly reduced under Parkinson's disease conditions in rats, coadministration of vitamin E with TiO_2-NPs raised the survival of brain cells (Jamali et al. 2023). In this study, the neuroprotective effect of quercetin in reducing neuron cell death was observed. This compound appears to have strong antioxidant effects against oxidative stress caused by Glut and Al, which is consistent with the findings of other studies (Sakanashi et al. 2008). Reducing necrosis subsequently eliminating inflammation and increasing vitality play a role in curing neurodegenerative illnesses. These results demonstrated that Glut-induced neurotoxicity in primary neuron culture was mostly due to ROS and resulted from the synergistic effects of quercetin in combination with Ce_2O_3NPs preventing such cell death and oxidative stress.

Conclusion

The results of the current study indicate that Q+Al+Ce_2O_3NP can ameliorate Glut-induced neurotoxicity, especially when used at a dose of 25 µg/ml. The combination of quercetin and Ce_2O_3NPs is suggested as a promising therapeutic method to alleviate Glut-induced neurotoxicity and suggests that the main mechanism involving this effect may be due to the antioxidant and anti-inflammatory properties of both agents. To support this finding, more studies are needed.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00210-024-03610-w.

Author contribution Y.Y. and S.G. made significant contributions to the general idea and design of the study. A.H. took part in the study. Y.Y. and H.N. completed the data analysis and article. All authors have read and approved the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

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Data availability The data supporting this study's findings are available from the corresponding author upon reasonable request.

Declarations

Ethical approval Animal procedures and protocols were carried out under the Animal Care and Use guidelines of Atatürk University Medical Experiment Application and Research Center. Approval was granted by the Ethics Committee of Ataturk University, Erzurum, Turkey (no: E-42190979-000-2300239665).

Informed consent N/A.

Conflict of interest The authors declare no competing interests.

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