Check for updates
Natural Product Sciences
31(1) : 49-54 (2025)
https://doi.org/10.20307/nps.2025.31.1.49

Therapeutic Potential of Methanol Extract of *Euonymus alatus* in HT22 Cells Through Neuroprotective Mechanisms

Youn Sik Jung[1], and Choong Je Ma[1,2,*]

[1]*Department of Medical Biomaterials Engineering, College of Biomedical Sciences, Kangwon National University, Chuncheon 24341, Republic of Korea*
[2]*Institute of Bioscience and Biotechnology, Kangwon National University, Chuncheon 24341, Republic of Korea*

Abstract – Oxidative stress, a key factor in the progression of brain diseases, induces apoptosis through multiple pathways. This study investigates the protective effects of *Euonymus elatus*, a traditional Korean remedy for conditions such as atherosclerosis, dysmenorrhea, and pain, on neuronal cells under oxidative stress. Excess glutamate was used to model oxidative stress, resulting in elevated reactive oxygen species (ROS), increased intracellular calcium (Ca^{2+}), reduced mitochondrial membrane potential, and decreased activity of glutathione-related enzymes, including glutathione reductase (GR) and glutathione peroxidase (GPx). Experimental results revealed that *E. elatus* extract provided significant cytoprotective effects. The extracts improved cell viability in MTT assays, reduced ROS and Ca^{2+} levels, restored mitochondrial membrane potential, and enhanced the activity of GR and GPx. These findings highlight the potential of *E. elatus* as a therapeutic candidate for mitigating oxidative stress and treating neurodegenerative disorders, such as Alzheimer's disease.
Keywords – *Euonymus alatus*, Alzheimer's disease, HT22 cell, Oxidative stress, Glutathione

Introduction

Alzheimer's Disease (AD) is the most common neurodegenerative disorder, characterized by progressive cognitive decline, memory impairment, and behavioral abnormalities.[1] AD is a multifactorial disease involving a complex interplay of biological and environmental factors. Key pathological hallmarks include abnormal accumulation of amyloid-beta (Aβ) plaques, formation of neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau proteins, neurotransmitter deficits, oxidative stress, and genetic predispositions.[2] Among the many contributors to AD, glutamate, the primary excitatory neurotransmitter in the central nervous system (CNS), plays a dual role. While essential for synaptic transmission, learning, and memory, glutamate dysregulation poses significant neurotoxic risks.[3,4] In pathophysiological conditions such as hypoxia, brain trauma, or ischemia, extracellular glutamate levels can rise to as much as 50 times their normal concentration. This glutamate excitotoxicity disrupts neuronal homeostasis, leading to severe neuronal damage.[5,6] Excess glutamate overstimulates NMDA (*N*-methyl-D-aspartate) receptors, resulting in calcium ion (Ca^{2+}) overload within neurons. Elevated intracellular Ca^{2+} triggers mitochondrial dysfunction, further increasing the production of reactive oxygen species (ROS). This cascade amplifies oxidative stress, a key driver of cellular apoptosis and neurodegeneration.[7] Additionally, chronic glutamate toxicity diminishes the synthesis and activity of glutathione (GSH), an essential antioxidant defense molecule composed of glutamate, cysteine, and glycine.[8] GSH protects cells by neutralizing ROS and maintaining redox balance. However, in glutamate-dominant oxidative stress environments, the activity of glutathione-related enzymes such as glutathione reductase (GR) and glutathione peroxidase (GPx) is significantly reduced.[9] This enzymatic decline compromises the cellular antioxidant system, leaving neurons vulnerable to damage.[10] The resulting mitochondrial dysfunction and oxidative damage contribute to synaptic loss, a hallmark of AD pathology.[10] The combined effects of glutamate-induced excitotoxicity, oxidative stress, and impaired antioxidant defenses exacerbate neuronal cell death, fostering the progression of AD. Given the pivotal roles of these mechanisms in AD pathogenesis, therapeutic strategies targeting glutamate regulation and oxidative

*Author for correspondence
Choong Je Ma, Ph. D., Department of Medical Biomaterials Engineering, College of Biomedical Science, Kangwon National University, Kangwondaehak-gil 1, Chuncheon 24341, Republic of Korea
Tel: +82-33-250-6565; E-mail: cjma@kangwon.ac.kr

stress hold promise for mitigating disease onset and progression. Approaches may include NMDA receptor antagonists, ROS scavengers, and compounds that enhance GSH synthesis or enzyme activity. In summary, understanding the intricate relationships between glutamate dysregulation, oxidative stress, sand neurodegeneration is critical for developing effective interventions to combat AD and other glutamate-mediated neurological disorders.

In Korea, *Euonymus alatus*, also known as winged euonymus, has a rich history of use in traditional medicine. It is employed for various purposes, including the prevention of atherosclerosis, the treatment of dysmenorrhea, the regulation of blood circulation, pain relief, and the elimination of stagnant blood.[11–14] The cork cambium found on the twigs of *E. alatus*, known in Korean traditional medicine as "Gui-Jun Woo," has been specifically recognized for its anticancer properties. This plant contains various bioactive compounds, including flavonoids such as quercetin, kaempferol, and rutin, as well as alkaloids like alatamine, eunymine, and evorine.[15–17] The antitumor potential of *E. alatus* has been highlighted in recent pharmacological studies utilizing a range of in vitro and in vivo models.[18] Nonetheless, there have been no reports on the neuroprotective activity of *E. alatus*.

In this study, we conducted a series of experiments to investigate the potential of *E. alatus* as a therapeutic and preventive agent for degenerative brain diseases, including AD. We explored its capacity to mitigate oxidative stress and inflammation, both of which are critical factors in the progression of AD and other neurodegenerative disorders.

Experimental

Plant materials and extract preparation – The dried *Euonymus alatus* herbs were sourced from the Kyungdong Traditional Herbal Market in Seoul, Korea. To extract the leaves, an 80% methanol solution was used, and the process was carried out in nine cycles of 60 minutes each with ultrasonic assistance. The extract was then concentrated under reduced pressure, resulting in a solid form. The voucher specimen (No. CJ090M) has been archived at the Natural Product Laboratory, Kangwon National University, Chuncheon, Korea.

Cell culture – The HT22 cell line, derived from the mouse hippocampus, was used to evaluate cell viability under glutamate-induced apoptosis. Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS) to provide necessary growth factors. Additionally, 1% penicillin/streptomycin was added to prevent bacterial contamination, and 2 mg/mL sodium bicarbonate ($NaHCO_3$) was included to help maintain the pH of the medium. The culture was further enriched with 15 mM 2-(4-(2-hydroxyethyl)-1-piperazinyl)-ethanesulfonic acid (HEPES) to buffer the solution and stabilize the pH. Cells were incubated at 37°C in a humidified incubator with a 5% CO_2 atmosphere to mimic physiological conditions. These conditions were optimal for the cultivation of HT22 cells for studying their response to glutamate-induced apoptosis.

Cell viability – HT22 cells were plated at a density of 1.7×10^4 cells per well in 48-well plates and cultured for 24 hours at 37°C in a humidified incubator with 5% CO_2. For the control and control groups, 300 μL of DMEM was added to each well. The positive control group was treated with 300 μL of Trolox. The test groups were treated with 300 μL of *E. alatus* extract at concentrations of 10, 20, and 50 μg/mL. After 1 hour of incubation, 3 mM glutamate was added to all wells except for the control group, which received an additional 300 μL of DMEM. The plates were further incubated for 23 hours. (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) solution (1 μg/mL) was then added to each well, followed by a 3-hour incubation. The MTT formazan was dissolved in dimethyl sulfoxide (DMSO), and the optical density (OD) at 570 nm was measured using an ELISA reader. The neuroprotective activity of the samples was calculated based on relative protection (%), using the following formula:

Relative protection (%) = [(OD of glutamate-treated with sample group − OD of glutamate-only group) / (OD of control group − OD of glutamate-only group)] × 100

Measurement of intracellular ROS levels – HT22 cells were cultured at 37°C and treated with glutamate, Trolox, and varying concentrations of *E. alatus* extract. After 8 hours, 40 μL of 100 μM 2′,7′-dichlorofluorescin diacetate (DCF-DA) was added to each well to measure reactive oxygen species (ROS) levels. The cells were incubated with DCF-DA for 1 hour, after which the medium was removed, and 300 μL of 1.0% Triton X-100 solution was added to lyse the cells. The plates were incubated at 37°C for 15 minutes to ensure complete lysis. Fluorescence of the cell lysate was then measured twice using a microplate reader, with excitation and emission wavelengths set to 490 and 525 nm, respectively. The resulting fluorescence intensity reflected the ROS levels in the cells.

Measurement of intracellular Ca^{2+} levels – HT22 cells were cultured and treated with Trolox and different concentrations of *E. alatus* extract. To evaluate intracellular calcium levels, 10 μL of 20 μM Fura-2 AM, a calcium-

sensitive dye, was added to each well. The cells were incubated at 37°C for 1 hour, allowing the dye to penetrate the cells and bind to intracellular calcium. Afterward, glutamate was introduced to induce calcium influx, and the cells were further incubated at 37°C for 2 hours. Following this, the medium was removed, and 150 µL of 1.0% Triton X-100 solution was added to lyse the cells. The plates were incubated for 15 minutes at 37°C to release the calcium-dye complex. Fluorescence intensity was measured using a microplate reader, with excitation set at 340 nm to detect the calcium-bound form of Fura-2 AM and emission at 510 nm to measure the calcium-free form.

Measurement of mitochondrial membrane potential – HT22 cells were cultured and treated with various concentrations of *E. alatus* extract prepared through serial dilution. To induce apoptosis, 3 mM glutamate was added, and the cells were incubated at 37°C for 1 hour. Mitochondrial membrane potential was assessed using Rhodamine 123, a dye specific to mitochondria. A 10 µL aliquot of the dye was added to each well, and the cells were incubated at 37°C for 30 minutes to allow the dye to accumulate in the mitochondria based on their membrane potential. After incubation, the cells were washed three times with PBS to eliminate excess dye and reduce background fluorescence. Fluorescence intensity was measured twice using a microplate reader, with excitation and emission wavelengths set to 480 and 525 nm, respectively.

Measurement of glutathione reductase and glutathione peroxides – HT22 cells were cultured and treated with glutamate, Trolox, and different concentrations of *E. alatus* extract. After 8 hours of incubation, the cells were washed three times with 0.2 M phosphate buffer (pH 7.4) to remove any residual substances. The washed cells were lysed using sulfosalicylic acid to ensure complete disruption. The lysates were then centrifuged at 3000 g for 30 minutes at 4°C, and the resulting supernatant was collected for enzymatic analysis. To evaluate glutathione reductase (GR) activity, the reduction of glutathione disulfide (GSSG) to reduced glutathione (GSH) was measured in the presence of NADPH, reflecting the enzyme's catalytic function. GR activity was quantified by monitoring the rate of GSSG reduction over time. Similarly, glutathione peroxidase (GPx) activity was determined by assessing the oxidation of GSH to GSSG. The extent of this oxidation was measured as a change in absorbance at 340 nm, providing a direct assessment of GR and GPx activities in the collected supernatant.

Statistical analysis – All experimental data are presented as mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, utilizing IBM SPSS Statistics software (version 26) and Microsoft Excel. Statistical significance was determined at p-values of < 0.05, < 0.01, and < 0.001, indicating differences between the experimental groups.

Results and Discussion

HT22 cells, a hippocampus-derived cell line commonly used in studies related to the central nervous system (CNS), were chosen as the experimental model. Glutamate and Trolox, both well-established agents in cytoprotective research, were utilized to investigate the protective effects of *E. alatus*. The elevation of glutamate levels is known to induce oxidative stress and apoptosis in neurons, making it an appropriate mechanism to assess cellular protection.[19] Neuronal cell death plays a critical role in the development of various cognitive impairments, including memory and learning deficits. The progressive loss of neurons can disrupt the brain's ability to form and retain new memories, leading to significant cognitive decline. This cellular damage is often associated with the onset of neurodegenerative diseases, such as AD and Parkinson's disease (PD), where the degeneration of specific neuronal populations contributes to the deterioration of mental functions and overall brain health.[20] We evaluated the neuroprotective effects of *E. alatus* against glutamate-induced neurotoxicity using the MTT assay. In HT22 cells, the extract demonstrated a protective effect against glutamate-induced cytotoxicity. Approximately 62.16% of cells exposed to glutamate damage remained viable compared to untreated cells. However, pretreatment with *E. alatus* at increasing con-

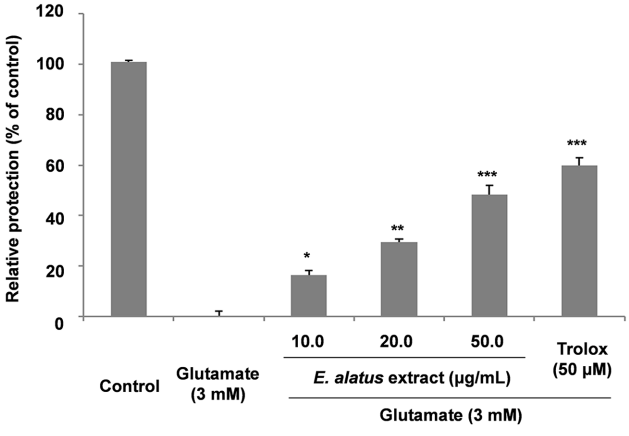

Fig. 1. The protective effect of *E. alatus* against glutamate-induced toxicity in HT22 cells. Data represents the mean ± S.D. of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to glutamate-treated cells.

centrations (10, 20, and 50 μg/mL) significantly enhanced cell viability in a dose-dependent manner. At concentrations of 10, 20 and 50 μg/mL, *E. alatus* displayed notable neuroprotective activity, with relative protective rates of 16.43%, 29.32% and 48.23%, respectively. Particularly, at 50 μg/mL, the extract exhibited the strongest neuroprotective effect, comparable to that of the positive control, Trolox (Fig. 1).

Excessive glutamate triggers the overproduction of reactive oxygen species (ROS) by overstimulating glutamate receptors, leading to calcium influx, mitochondrial dysfunction, and oxidative stress. This process damages cellular components and contributes to neuronal cell death, playing a key role in neurodegenerative diseases.[7] The inhibition of ROS production was assessed using fluorescence measurements. In glutamate-treated cells, fluorescence increased to 152.01% compared to the control group, indicating a significant rise in ROS levels. However, pretreatment with *E. alatus* extract effectively suppressed ROS production. At a concentration of 50 μg/mL, the extract reduced ROS levels to 108.63% of the untreated control (Fig. 2). These findings suggest that *E. alatus* exerts a protective effect on HT22 cells against glutamate-induced cytotoxicity by inhibiting ROS generation. The antioxidative effect of *E. alatus* at the concentration of 50 μg/mL was comparable to that of Trolox, a well-known antioxidant. In the human body, reactive oxygen species (ROS) are generated in response to various internal and external stresses, including metabolic activities and environmental factors. Excessive ROS in the brain disrupts cellular balance, leading to oxidative stress, which is a critical factor in the progression of neurodegenerative diseases such as AD.[21] The ability of *E. alatus* to mitigate ROS suggests its potential in protecting neuronal cells from oxidative damage and related degenerative conditions.

Abnormal intracellular Ca^{2+} levels lead to excessive ROS production, triggering oxidative stress and lipid peroxidation. This damages cell membranes and contributes to neurodegenerative diseases.[22] Glutamate activates NMDA receptors and Ca^{2+} channels, causing excessive Ca^{2+} influx that disrupts cellular homeostasis, leading to mitochondrial dysfunction, ROS production, and ultimately cell death.[23] Therefore, we assessed the effect of *E. alatus* extract on the changes in intracellular calcium ion concentrations, which are crucial in mediating cellular responses to stress and injury. We measured intracellular Ca^{2+} levels using Fura-AM staining. Glutamate treatment resulted in a significant increase in Ca^{2+} levels, reaching 157.62% of the control group. However, pretreatment with *E. alatus* extract led to a significant reduction in Ca^{2+} levels. At concentrations of 10, 20, and 50 μg/mL, *E. alatus* reduced Ca^{2+} levels to 139.41%, 122.12%, and 109.87% of the untreated control, respectively (Fig. 3). These findings suggest that *E. alatus* provides significant protection to HT22 cells from glutamate-induced cytotoxicity by modulating intracellular Ca^{2+} levels. By reducing the excessive influx of Ca^{2+}, *E. alatus* helps restore calcium homeostasis within the cells.

The mitochondrial membrane potential plays a significant role in determining cell fate, being closely associated with processes like necrosis and apoptosis. Consequently, numerous researchers are investigating the connection between mitochondrial function and cell survival. Notably, glutamate rapidly induces a decline in mitochondrial membrane potential.[24,25] Therefore, we assessed the effect of *E. alatus* extract on the changes in mitochondrial membrane potential,

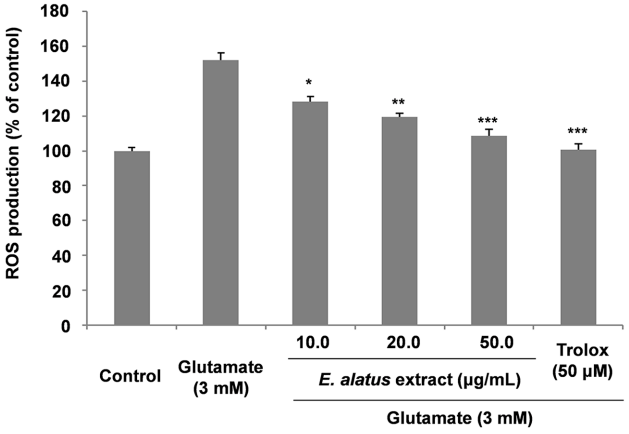

Fig. 2. The effect of *E. alatus* on intracellular ROS levels against glutamate toxicity in HT22 cells. Data represents the mean ± S.D. of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to glutamate-treated cells.

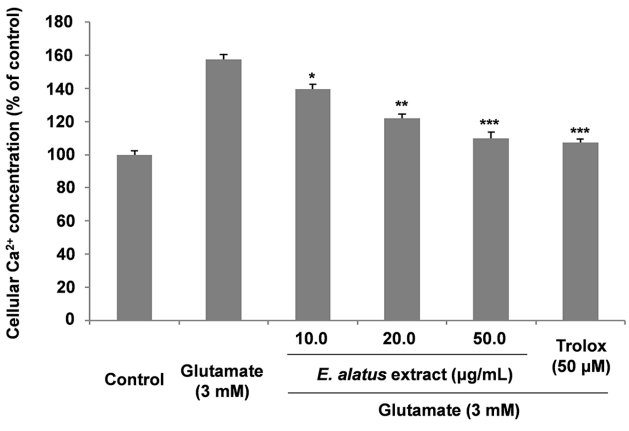

Fig. 3. The effect of *E. alatus* on intracellular Ca^{2+} amount against glutamate toxicity in HT22 cells. Data represents the mean ± S.D. of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to glutamate injured cells.

which are crucial in associating cellular death via necrosis and/or apoptosis. Cellular mitochondrial membrane potential levels were measured using rhodamine 123 staining. Our experiment demonstrated that *E. alatus* exhibits a cytoprotective effect by mitigating the mechanisms responsible for glutamate-induced depolarization of mitochondrial membrane potential. Glutamate reduced mitochondrial membrane potential by 70.13%. However, in cells treated with Trolox, the mitochondrial membrane potential was restored to 93.18%, a level comparable to that of the control group. Additionally, *E. alatus* increased mitochondrial membrane potential in a dose-dependent manner. At a concentration of 10 μg/mL, the mitochondrial membrane potential increased to 82.74%. At 20 μg/mL, it increased to 86.00%, and at 50 μg/mL, it reached 89.17% (Fig. 4).

A reduction in glutathione reductase (GR) and glutathione peroxidase (GPx) depletes glutathione, impairing cysteine uptake into cells and ultimately leading to neuronal cell death. Glutamate contributes to this process by decreasing GR and GPx levels and inhibiting glutathione synthesis.[26] We investigated the effects of *E. alatus* on the activity of glutathione reductase and glutathione peroxidase. Glutathione reductase and glutathione peroxidase are critical enzymes involved in glutathione production. Excessive glutamate reduces the activity of these enzymes. In the results, *E. alatus* showed a weak effect on the activity of both GR and GPx at low concentrations. However, as the concentration increased, the activity of these enzymes was significantly enhanced. These findings suggest that *E. alatus* effectively enhances GR and GPx activity under glutamate-induced stress, thereby preventing glutathione depletion. In cells treated with glutamate, GR and GPx activities decreased to 48.95% and 44.76% of control levels, respectively. Treatment with *E. alatus* in HT22 cells increased GR activity to 80.59%. Additionally, the reduced GPx activity caused by glutamate was significantly recovered by *E. alatus*, reaching 76.30% of control levels

In conclusion, this study demonstrates that *E. alatus* possesses neuroprotective properties. Our findings showed reductions in ROS and Ca^{2+} levels, increased mitochondrial membrane potential, and enhanced activities of GR and GPx. Previous research has revealed that *E. alatus* contains flavonoids such as quercetin, kaempferol, and rutin.[16] These flavonoids, which are secondary metabolites found in plants and fungi, are well-known for their potent antioxidant properties and are likely contributors to the effects observed in this study.[27] Overall, the results indicate that *E. alatus* has significant potential for preventing and treating neurodegenerative diseases by reducing oxidative stress and protecting neuronal cells from apoptosis.

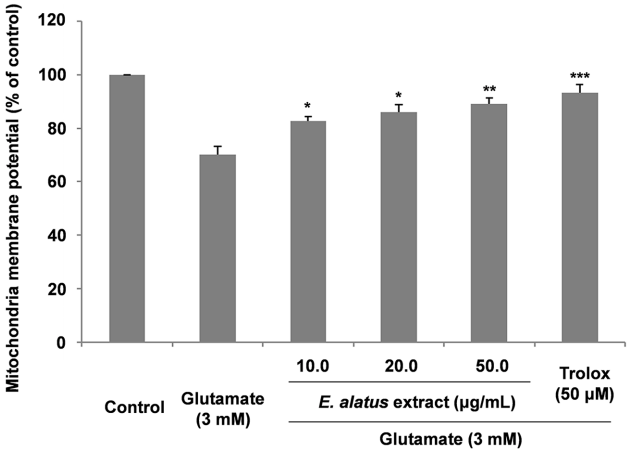

Fig. 4. The effect of *E. alatus* on mitochondrial membrane potential level against glutamate toxicity in HT22 cells. Data represents the mean ± S.D. of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to glutamate injured cells.

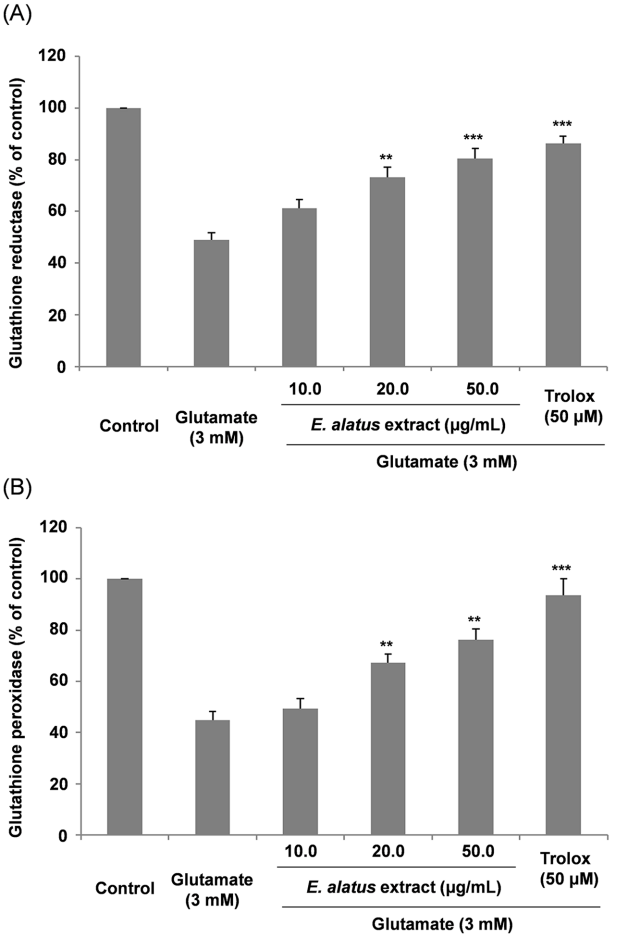

Fig. 5. The effect of *E. alatus* on GR and GPx enzyme against glutamate toxicity in HT22 cells. Data represents the mean ± S.D. of three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared to glutamate injured cells.

Conflict of Interest

The authors have declared no conflict of interest.

Acknowledgement

This research was supported by Korea Basice Science Institute (National Research Facilities and Equipment Center) grant funded by the Ministry of Education (grant No. 2022R1A6C101A739)

References

(1) Breijyeh, Z.; Karaman, R. *Molecules* **2020**, *25*, 5789.
(2) O'Brien, R. J.; Wong, P. C. *Annu. Rev. Neurosci.* **2011**, *34*, 185–204.
(3) Song, J.; Yang, X.; Zhang, M.; Wang, C.; Chen, L. *J. Alzheimers Dis.* **2021**, *84*, 557–578.
(4) Puranik, N.; Song, M. *Molecules* **2024**, *29*, 5744.
(5) Verma, M.; Lizama, B. N.; Chu, C. T. *Transl. Neurodegener.* **2022**, *11*, 3.
(6) Johnston, M. V. *Brain Pathol.* **2005**, *15*, 234–240.
(7) Zhang, H.; Jiang, X.; Ma, L.; Wei, W.; Li, Z.; Chang, S.; Wen, J.; Sun, J.; Li, H. *Front. Cell Dev. Biol.* **2022**, *10*, 964075.
(8) Lewerenz, J.; Dargusch, R.; Maher, P. *J. Neurochem.* **2010**, *113*, 502–514.
(9) Pereira, C. M.; Oliveira, C. R. *Free Radic. Biol. Med.* **1997**, *23*, 637–647.
(10) Bornstein, R.; Mulholland, M. T.; Sedensky, M.; Morgan, P.; Johnson, S. C. *Mol. Cell. Neurosci.* **2023**, *126*, 103887.
(11) Zhai, X.; Lenon, G. B.; Xue, C. C.; Li, C.-G. *Evid. Based Comp. Alt. Med.* **2016**, *2016*, 9425714.
(12) Gurung, P.; Lim, J.; Magar, T. B. T.; Shrestha, R.; Kim, Y.-W. *Antioxidants* **2024**, *13*, 433.
(13) You, Y.-L.; Byun, H.-J.; Lee, J. S.; Choi, H.-S.; Youk, J.-S. *Food Sci. Biotechnol.* **2024**, *33*, 3567–3577.
(14) Chang, B.; Jin, C.; Zhang, W.; Kong, L.; Yang, J.-H.; Lian, F.-M.; Li, Q.-F.; Yu, B.; Liu, W.-K.; Yang, L.-L.; Zhao, P.; Zhen, Z. *Am. J. Chin. Med.* **2012**, *40*, 1177–1187.
(15) Yoon, W.-J.; Min, H.-J.; Cho, H.-D.; Kim, H.-G.; Park, W.-L.; Kim, D.-H.; Tachibana, H.; Seo, K.-I. *Biomedicines* **2024**, *12*, 2928.
(16) Middleton, E.; Kandaswami, C. *Biochem. Pharmacol.* **1992**, *43*, 1167–1179.
(17) Kitanaka, S.; Takido, M.; Mizoue, K.; Nakaike, S. *Chem. Pharm. Bull.* **1996**, *44*, 615–617.
(18) Kim, C.-H.; Kim, D.-I.; Kwon, C.-N.; Kang, S.-K.; Jin, U.-H.; Suh, S.-J.; Lee, T.-K.; Lee, I.-S. *Int. J. Gynecol. Cancer* **2006**, *16*, 843–848.
(19) Prasad, K. N.; Bondy, S. C. *Mech. Ageing Dev.* **2020**, *185*, 111191.
(20) Moonen, S.; Koper, M. J.; Van Schoor, E.; Schaeverbeke, J. M.; Vandenberghe, R.; Von Arnim, C. A. F.; Tousseyn, T.; De Strooper, B.; Thal, D. R. *Acta Neuropathol.* **2023**, *145*, 175–195.
(21) Ionescu-Tucker, A.; Cotman, C. W. *Neurobiol. Aging* **2021**, *107*, 86–95.
(22) Cascella, R.; Cecchi, C. *Int. J. Mol. Sci.* **2021**, *22*, 4914.
(23) Ashok, D.; Papanicolaou, K.; Sidor, A.; Wang, M.; Solhjoo, S.; Liu, T.; O'Rourke, B. *J. Biol. Chem.* **2023**, *299*, 104708.
(24) Samanta, S.; Akhter, F.; Roy, A.; Chen, D.; Turner, B.; Wang, Y.; Clemente, N.; Wang, C.; Swerdlow, R. H.; Battaile, K. P.; Lovell, S.; Yan, S. F.; Yan, S. S. *Brain* **2024**, *147*, 1710–1725.
(25) Area-Gomez, E.; Schon, E. A. *Curr. Opin. Genet. Dev.* **2016**, *38*, 90–96.
(26) Gao, Y.; Li, J.; Wu, Q.; Wang, S.; Yang, S.; Li, X.; Chen, N.; Li, L.; Zhang, L. *Int. Immunopharmacol.* **2021**, *99*, 108002.
(27) Nakajima, A.; Ohizumi, Y. *Int. J. Mol. Sci.* **2019**, *20*, 3380.

Received January 13, 2025
Revised February 28, 2025
Accepted March 12, 2025