The European Research Journal 2022;8(4):521-528 Original Article

DOI: 10.18621/eurj.1027423 *Biophysics*

Quipazine treatment exacerbates oxidative stress in glutamate-induced HT-22 neuronal cells

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ABSTRACT

Objectives: Quipazine is a serotonin agonist. It is known that serotonin, an important neurotransmitter, contributes to the etiology of psychiatric and many neurodegenerative diseases. However, the effect of the serotonin agonist quipazine on HT-22 cells in glutamate-induced cytotoxicity is unknown. This study aims to investigate the effect of quipazine on increased oxidative stress (OS) as a result of glutamate-induced cytotoxicity in HT-22 cells.

Methods: The cells were divided into 4 groups, Control group: no treatment was applied, Glutamate group: glutamate was incubated at 10 mM for 24 h, Quipazine group: incubated with different doses of quipazine for 24 h, Quipazine+Glutamate group were pre-treated with various concentrations (25, 50, 100 and 200 µM) of quipazine for 1 h and then exposed to 10 mM glutamate for 24 h. Cell viability rate between groups was measured by the XTT assay. OS and antioxidant levels were measured with the Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) Elisa kits, and Caspase-3 levels were also examined in caspase activity.

Results: Quipazine at different concentrations showed significant differences in cell viability in HT-22 cells. An appropriate dose of 25 µM was accepted for quipazine in the study. Quipazine treatment with glutamate-toxicity in the cells further reduced TAS levels and significantly increased TOS levels. It was also observed that the Caspase-3 level increased more in the Quipazine + Glutamate group according to the Glutamate group.

Conclusions: The results determined that the use of quipazine is an agent that will further increase the neurodegeneration caused by glutamate toxicity.

Keywords: Quipazine, glutamate-toxicity, oxidative stress, HT-22 cell, cell viability

It is now accepted that excitotoxicity has an important role in the etiology of neurodegenerative diseases. However, the mechanisms of excitotoxicity in the neurodegeneration process are still not fully understood and need further research [1]. Excitotoxicity was first introduced in the 1960s and describes neurodegeneration that occurs as a result of excessive or prolonged activation of amino acid receptors responsible for stimulation [2]. From this overstimulation; more than normal neurotransmitters release into the synaptic gap, failure of the pumps that take neurotransmitters back from the synapse, or overexpression of the receptors in the post-synaptic neuron may be responsible [3]. Oxidative stress (OS) has been reported as the most important damage mechanism in glutamate excitotoxicity [4]. Especially in neuronal cells, receptor activation due to glutamate density causes calcium ion entry into the cell, causing mitochondrial dysfunction and excessive reactive oxygen species (ROS) formation. An excessive increase in intracellu-

Received: *November 23, 2021;* ***Accepted:*** *April 12, 2022;* ***Published Online:*** *May 18, 2022*

How to cite this article: *Yıldızhan K, Öztürk A. Quipazine treatment exacerbates oxidative stress in glutamate-induced HT-22 neuronal cells. Eur Res J 2022;8(4):521-528. DOI: 10.18621/eurj.1027423*

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e-ISSN: 2149-3189

lar ROS also results in neurodegeneration [5, 6].

Glutamate is a neurotransmitter that is important for maintaining the balance of excitatory and inhibitory neurons in the central nervous system [7]. In general, it is responsible for cognitive, motor, sensory, and autonomic activity with its stimulating function. Many physiological cases as diversification, cell migration, synapse induction, and death also play an essential role. Because of these important tasks, it is very important to keep the glutamate level at a certain level. Studies have shown that abnormalities in glutamate levels have been associated with many neurodegenerative diseases [8, 9]. However, there is no established mechanism for toxicity from glutamate overdose, and some believe this process may be mediated through differential activation and inhibition of certain receptors in the cell [10].

Serotonin plays an important role in the initiation and modulation of locomotor behaviour in mammals [11]. In some studies, it has been stated that serotonin activates some cell signaling pathways and decreases intracellular OS damage [12-14]. Quipazine is a serotonin (5-HT) agonist [15]. It has been reported that it has a potential agonist activity for 5-HTR1b [16]. However, quipazine also acts as a potent antagonist on peripheral 5-HT3 receptors [17].

It is known that glutamate-induced cytotoxicity causes neuronal damage in brain cells. Therefore, important to develop a protective therapeutic approach against glutamate-induced excitotoxicity of cells, as well as to investigate the events that will trigger this damage. In our study, we examined the effect of quipazine-mediated serotonin activation on intracellular oxidative stress and caspase activation in a glutamate toxicity model in the HT-22 neuronal cell line.

METHODS

Cell Culture

The HT-22 cell line (SCC129) was selected in this investigation [18]. The Merck provided HT-22 mouse

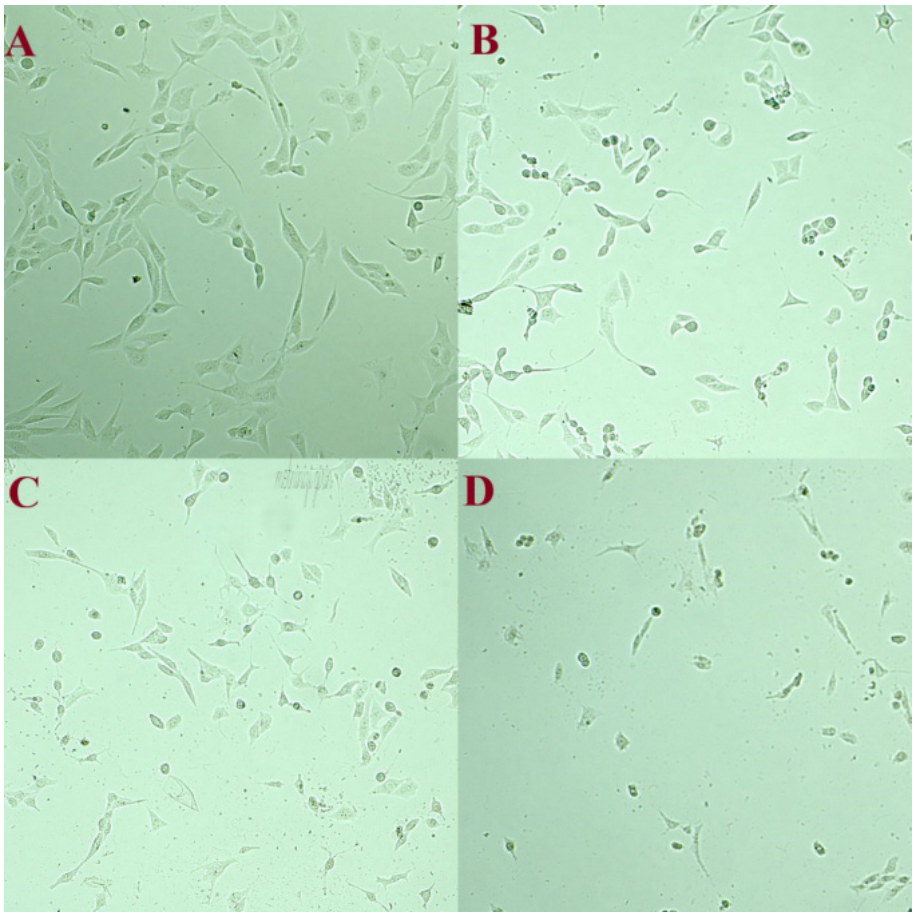

Fig. 1. Images of cells in groups under the microscope. A) Control, B) Quipazine (25 µM), C) Glutamate (10 mM), and D) Quipazine + Glutamate.

hippocampal neuronal cell lines. The cells were cultured in DMEM (Thermo Fisher Scientific, Altrincham, UK) containing 10% Fetal Bovine Serum and 1% L-glutamine and 1% penicillin-streptomycin (Sigma-Aldrich Co., USA). The HT-22 cells were incubated at 37° C and 5% CO_2.

Study Groups

The cells were divided into 4 groups and were prepared to evaluate the effect of quipazine on glutamate-induced cytotoxicity: 1)-Control group: no application has been made. 2)- Glutamate group: glutamate was incubated at 10 mM for 24 h [19]. 3)-Quipazine group: incubated with different doses of quipazine for 24 h. 4)-Quipazine+Glutamate group were pre-treated with different concentrations (25, 50, 100 and 200 µM) of quipazine for 1 h and then exposed to 10 mM glutamate for 24 h (Fig. 1).

Drug Administration

Quipazine dimaleate (Cat no:0629, Tocris) and glutamate (Sigma-Aldrich Co., USA) were dissolved in the cell medium.

Cell Viability Assay

After the doses and duration that we specified for the study are completed, XTT (Abcam, UK) analysis was performed to determine cell viability in the cells. HT-22 mouse hippocampal (SCC129) cells were seeded in 96 wells at 1 x 10^4 cell density in 0.1 mL of DMEM. Different dose ranges (25, 50, 100 and 200 µM) for Quipazine were added to the plate wells. After 1 hour, when the incubation period expired, 10 mM glutamate was added to each well and incubated for a total of 24 h. At the end of the total time (24 h), the XTT (50 µL) reagent mixture was added to the HT-22 cells in the plate wells. In the XTT incubation, the cells were incubated for 4 h in the incubator (37°C and 5% CO2). The absorbance value for XTT analysis was gauged at 450 nm using a microplate reader (Multiskan PLUS, Thermo Scientific For all tests, three replicate readings were made. XTT analysis results are shown as the percentage of viable cells in comparison with the Control group, which did not receive any chemicals.

Preparation of Cells Homogenates

When the indicated incubation times expired, the cells in the plate were transferred to sterile Eppendorf tubes. Cells in the eppendorf tubes were centrifuged at 2000 rpm for 10 min. Supernatants were carefully removed from Eppendorf tubes in laminar flow. Cell pellets under Eppendorf were diluted through phosphate buffered saline (pH: 7.4) to a concentration of 1 million/ml. The cell structure was lysed by repeated freeze-thaw and the intracellular components were permitted to recede. The resulting mixture was centrifuged at 4000 rpm for 10 min. The supernatant remaining in the upper part of the tubes was taken with the help of sterile pipettes and transferred to different sterile tubes for biochemical analysis. Total protein levels for each group were measured with the Bradford assay kit. (Merck, Germany).

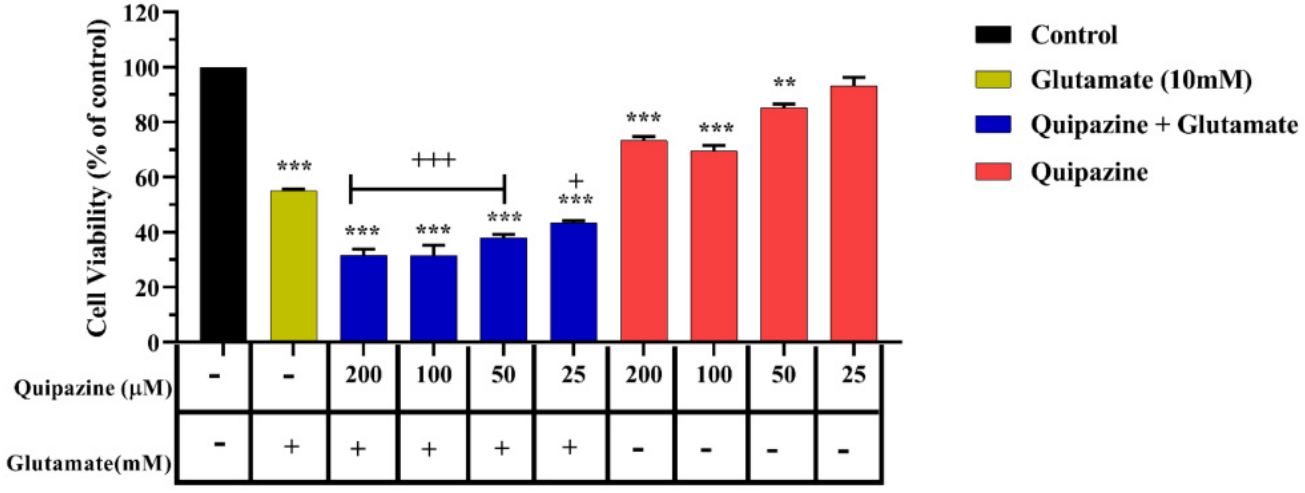

Fig. 2. Effect of Quipazine on glutamate-induced HT-22 cells. (Data are expressed as mean ± mean standard error). (* $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ compared to the control group; +++ $p < 0.001$ and + $p < 0.01$ compared with glutamate group).**

Measurement of Total Antioxidant Status (TAS), Total Oxidant Status (TOS) and Caspase-3 Levels in the HT-22 Cells

TAS, TOS, and Caspase-3 levels in the resulting supernatant of glutamate toxicity induced by HT-22 cells were determined using ELISA commercial kits (BT Lab, China). For the analysis, the protocols determined by the company for commercial kits were performed. Read at 450 nm in an microplate reader (Thermo-Fisher Scientifc, UK).

Statistical Analysis

The results were expressed as mean ± standard error (SEM). SPSS Version 23.0 for Windows was used to analyze the data. ANOVA was used to analyze the data, and the post-hoc Tukey test was used to determine the differences between the groups. A statistically significant result of $p < 0.05$ was accepted.

RESULTS

Effect of Quipazine on Cell Survival in Glutamate-Induced HT-22 Cells

The cell viability was investigated in groups by incubating HT-22 cells with significant doses. XTT assay kit was used to determine cell viability. Cell viability for quipazine was determined in HT-22 cells treated with both control and glutamate at various doses (25, 50, 100, and 200 M). The cells were pretreated with increasing doses of Quipazine (25-200 µM) for 1 hour at first, and then they were incubated with or without glutamate (10 mM) for the next 24 h. As shown in Fig. 2, the 25 µM dose for quipazine did not appear to affect cell viability compared to the control group, and this dose was determined as the appropriate dose for the study. It was observed that the administration of a 25 µM Quipazine dose in the glutamate toxicity groups significantly decreased the cell viability rate ($p < 0.01$). In the Quipazine+Glutamate groups, it was observed that the cell viability rate decreased as the dose of Quipazine increased ($p < 0.001$) (Fig. 2).

Effect of Quipazine on TAS Level in Glutamate-Induced HT-22 Cells

In the groups formed, the effect of quipazine was measured against glutamate cytotoxicity with the TAS Elisa kit in the cells. Compared to the control group, the TAS level of the Glutamate group was significantly lower ($p < 0.01$). Compared to the control group, the TAS level of the Quipazine group was considerably lower ($p < 0.05$). In the Quipazine+Glutamate group, the TAS level was determined that it is dramatically lower than both the Control group and the Glutamate group ($p < 0.01$, $p < 0.001$) (Fig. 3).

Effect of Quipazine on TOS Level in Glutamate-Induced HT-22 Cells

In the groups formed, the effect of quipazine was measured against glutamate cytotoxicity with the TOS Elisa kit in the cells It was observed that the TOS level of the Glutamate group increased considerably com-

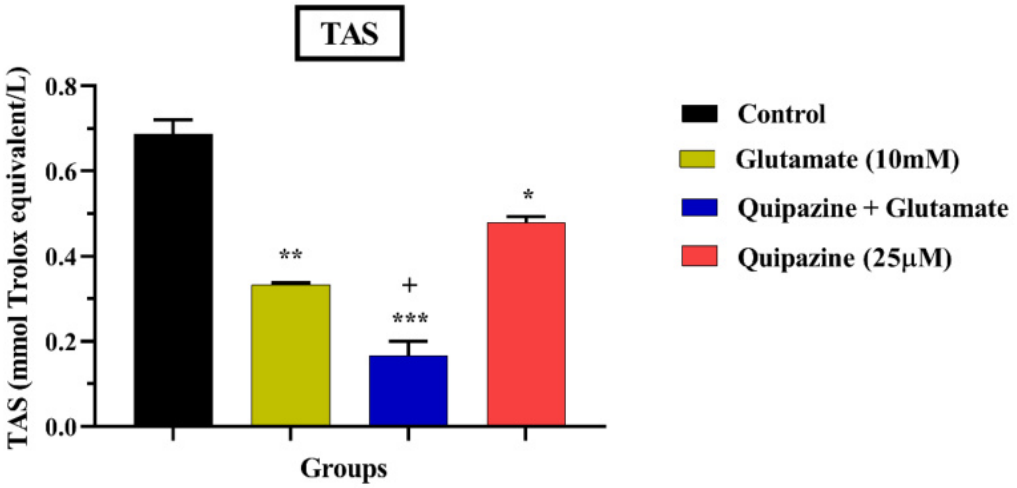

Fig. 3. Effect of Quipazine on TAS level in HT-22 cells. (Data are expressed as mean ± mean standard error). (* $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ compared to the control group; + $p < 0.01$ compared with glutamate group). TAS = Total Antioxidant Status**

pared to the control group ($p < 0.01$). In the Quipazine+Glutamate group, the TOS level was found to be significantly higher than in both the Control group and the Glutamate group ($p < 0.001$, $p < 0.01$) (Fig. 4).

Effect of Quipazine on Caspase-3 Level in Glutamate-Induced HT-22 Cells

ELISA commercial kits were used to investigate the effect of quipazine on Caspase-3 levels in glutamate-induced HT-22 cells. The Caspase-3 level was significantly increased between the groups when the Quipazine+Glutamate group was compared to the control and Quipazine groups ($p < 0.001$). There was no statistically significant difference between the control group and the Quipazine group ($p > 0.05$). When the Quipazine+Glutamate group was compared to the Quipazine group between the groups, it was observed that the Caspase-3 level increased significantly in the Quipazine+Glutamate group ($p < 0.001$) (Fig. 5).

DISCUSSION

In this study, the effect of pretreatment of different doses of quipazine against glutamate-induced toxicity was investigated. It was observed that pretreatment with quipazine further increased oxidative stress and reduced antioxidant level against glutamate-induced cytotoxicity in HT-22 cells. We also determined that it increased the activation of Caspase-3, which is an important marker in the apoptotic pathway.

Studies have shown that administration of the nighttime serotonin agonist quipazine to rats delays the rhythms of melatonin metabolite excretion and activity similar to light [20, 21]. Serotonin has a crucial effect on the initiation and modulation of locomotor behaviour in mammals [11]. In a study, it was stated that serotonin does not protect C6 cells from glutathione depletion by glutamate. Incubation of serotonin and glutamate together depletes the cellular glutathione level. Serotonin caused a significant inhibition of lipid peroxide accumulation in C6 glioma cells against glutamate exposure and controlled a low lipid peroxide accumulation rate [22]. A study using a serotonergic derivative of quipazine found promising results against skin cancer. It has been shown that the quipazine derivative can inhibit cellular growth by inducing S-phase cell cycle delay, ROS generation and apoptosis in cells [23]. In a study investigating the suppressive effect of N-palmitoyl serotonin on glutamate-induced apoptosis in HT-22 cells. It has been stated that N-palmitoyl serotonin promotes BDNF formation and secretion and then protects neuronal cells against oxidative stress-induced apoptosis through activation of the TrkB/CREB pathway [24]. In a previous study, we observed that TAS levels decreased and TOS, NO, and TNF-a levels increased in C6 cells after glutamate treatment in the cytotoxicity model we studied in Glutamate-induced C6 glia cells [25]. We hoped that the quipazine we used would reduce glutamate-induced cytotoxicity. However, in our study, after choosing the appropriate dose (25 µM) for quipazine, it was observed how it would affect glutamate toxicity. The re-

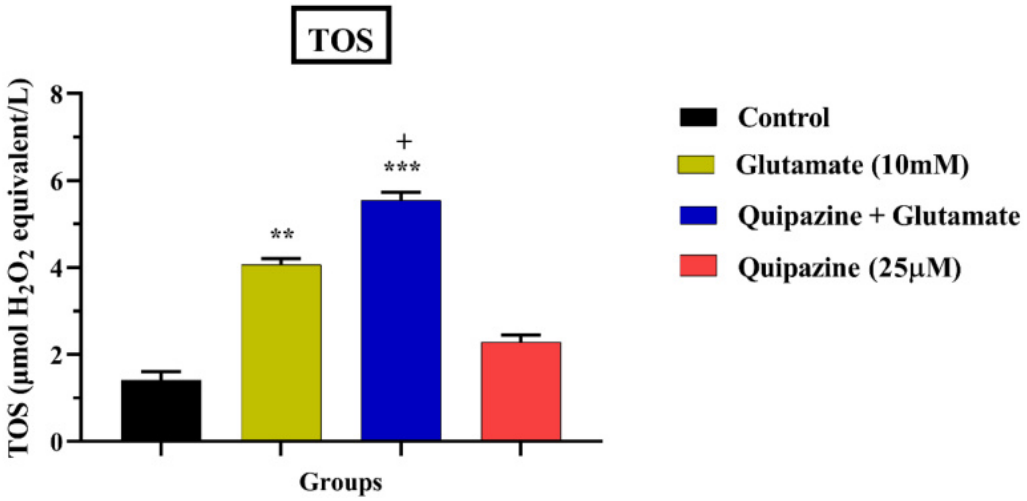

Fig. 4. Effect of Quipazine on TOS level in HT-22 cells. (Data are expressed as mean ± mean standard error). (* $p < 0.001$ and ** $p < 0.01$ compared to the control group; +$p < 0.01$ compared with glutamate group). TOS = Total Oxidant Status**

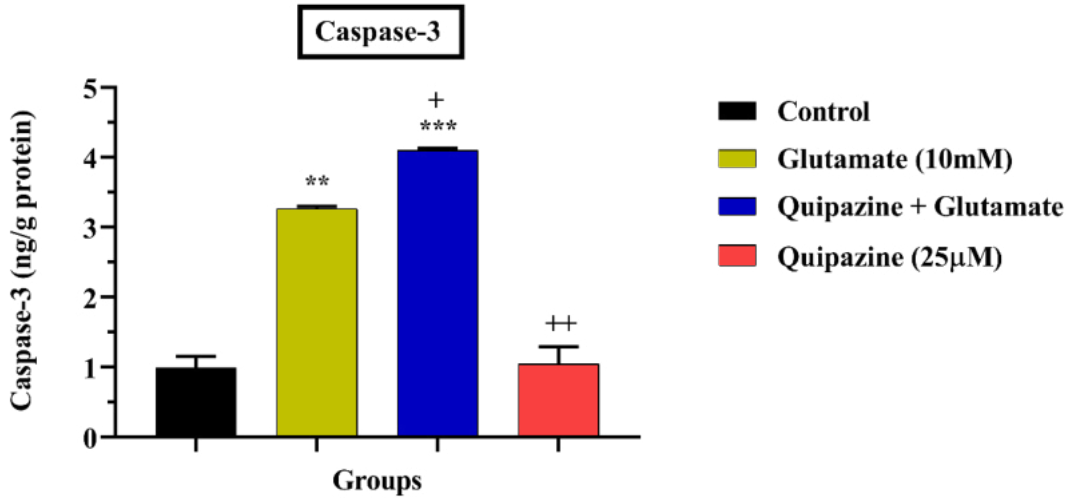

Fig. 5. Effect of Quipazine on Caspase-3 level in HT-22 cells after glutamate-induced cytotoxicity. (Data are expressed as mean ± mean standard error). (* $p < 0.001$, ** $p < 0.01$ compared to the control group; ++ $p < 0.001$ and + $p < 0.01$ compared with glutamate group).**

sults showed that even the use of an appropriate dose of quipazine decreases intracellular antioxidant capacity in HT-22 cells and increases intracellular oxidative stress and Caspase-3 activation (Figs. 3-5).

Glutamate is the most abundant excitatory neurotransmitter in the mammalian CNS. In the CNS, glutamate is found in many cell types and intracellular organelles [26]. Considering the etiology of neurodegenerative diseases, it is seen that neuronal damage follows a progressive process and first results in loss of function and finally death of the neuron [27]. At present, the damage mechanism of many neurodegenerative diseases is still elucidated, but there is increasing evidence that the pathogenesis of neurodegenerative diseases is associated with excitotoxin and oxidative stress [28, 29]. Glutamate is a substantial cause of nerve cell damage and is known to mediate oxidative and excitatory toxicity [30, 31]. For these reasons, it is important to find sources of oxidative stress and stimulant toxicity from glutamate toxicity. A recent study showed that "Kaempferia parviflora" extract, as a pharmacological agent against glutamate-induced cytotoxicity of HT-22 neuronal cells, regulates the increased level of oxidative stress in cells and therefore reduces apoptotic cell death [32]. In our study, we investigated the effects of glutamate toxicity and serotonin activation on HT-22 cells. Glutamate-induced excitotoxicity has an important role in the etiology of neurodegenerative diseases. Currently, there is no safe and effective drug to prevent glutamate-induced excitotoxicity [26]. OS have the most important role in the biochemical processes that lead to cell death in glutamate-induced excitotoxicity. In fact, cells can prevent OS damage thanks to the presence of various molecules that work as antioxidants. However, sometimes oxidative stress is observed in cells that do not have enough antioxidant capacity [7]. It has been emphasised that OS, which occurs with glutamate increase, is an important trigger for neurodegeneration [19, 33]. In addition, it is an important damage factor not only in Alzheimer's and Parkinson's disease, but also in neurodegenerative diseases that occur in living things. Studies have shown that the damage mechanisms of neuronal cells are associated with an increase in OS [19, 33, 34]. In a study, it was reported that melatonin and serotonin have effective DMPD radical scavenging activity and the ability to reduce copper ions. Serotonin has a phenolic hydroxyl group, so it has been emphasized that serotonin has higher radical scavenging and reducing activity than melatonin [35]. Unfortunately, with the results of this study (Figs. 3-5), we observed that serotonin activation, via the quipazine agonist, increased oxidative stress caused by glutamate toxicity.

CONCLUSION

The findings of this research showed that the use of an appropriate dose of quipazine increased cellular damage resistance to glutamate-induced cytotoxicity in HT-22 cells. We think that this damaging impact of

quipazine occurs with the activation of oxidative stress pathways, but more studies should be done to make this determination. Therefore, we determined that the use of appropriate doses of quipazine exacerbates glutamate toxicity that may occur in CNS disorders and causes more damage in neurodegeneration due to neuronal damage. However, further in vitro and in vivo studies are needed to answer questions about the possible mechanisms of action of quipazine in glutamate toxicity.

Authors' Contribution

Study Conception: KY; Study Design: KY, AO; Supervision: KY; Funding: KY, AO; Materials: N/A; Data Collection and/or Processing: AO; Statistical Analysis and/or Data Interpretation: KY, AO; Literature Review: KY, AO; Manuscript Preparation: KY and Critical Review: KY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during the conduction or writing of this study.

Acknowledgement

The authors would like to thank the CUTFAM Research Center, Sivas Cumhuriyet University, School of Medicine, Sivas, Turkey, for providing the necessary facilities to conduct this study.

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