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# Dexmedetomidine Attenuates Glutamate-Induced Cytotoxicity by Inhibiting the Mitochondrial-Mediated Apoptotic Pathway

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Data Interpretation D  
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**Background:** Glutamate (GLU) is the most excitatory amino acid in the central nervous system and plays an important role in maintaining the normal function of the nervous system. During cerebral ischemia, massive release of GLU leads to neuronal necrosis and apoptosis. It has been reported that dexmedetomidine (DEX) possesses anti-oxidant and anti-apoptotic properties. The objective of this study was to investigate the effects of DEX on GLU-induced neurotoxicity in PC12 cells.

**Material/Methods:** PC12 cells were treated with 20 mM GLU to establish an ischemia-induced injury model. Cell viability was accessed by MTT assay. MDA content and SOD activity were analyzed by assay kits. Apoptosis rate, ROS production, intracellular Ca<sup>2+</sup> concentration, and MMP were evaluated by flow cytometry. Western blot analysis was performed to analyze expressions of caspase-3, caspase-9, cyt-c, bax, and bcl-2.

**Results:** PC12 cells treated with GLU exhibited reduced cell viability and increased apoptosis rates, which were ameliorated by pretreatment with DEX. DEX significantly increased SOD activity, reduced content of MDA, and decreased production of ROS in PC12 cells. In addition, DEX clearly reduced the level of intracellular Ca<sup>2+</sup> and attenuated the decline of MMP. Moreover, DEX notably reduced expressions of caspase-3, caspase-9, cyt-c, and bax and increased expression of bcl-2.

**Conclusions:** Our findings suggest that DEX can protect PC12 cells against GLU-induced cytotoxicity, which may be attributed to its anti-oxidative property and reduction of intracellular calcium overload, as well as its ability to inhibit the mitochondria-mediated apoptotic pathway.

**MeSH Keywords:** **Apoptosis • Dexmedetomidine • Glutamic Acid • Mitochondria • Neuroprotective Agents • PC12 Cells**

**Abbreviations:** **DEX** – dexmedetomidine; **GLU** – glutamate; **DMEM** – Dulbecco's modified Eagle's medium; **MTT** – methyl thiazolyl tetrazolium; **MDA** – malondialdehyde; **SOD** – superoxide dismutase; **ROS** – reactive oxygen species; **NMDA** – n-methyl-d-aspartic acid; **MMP** – mitochondrial membrane potential; **Cyt-c** – cytochrome C; **AIF** – apoptosis inducing factor; **Apaf-1** – apoptotic protease activating factor-1; **PI3K** – phosphatidylinositol-3 kinase; **PKB** – protein kinase B; **FAK** – focal adhesion kinase

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/922139>

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## Background

Cerebrovascular disease is a common disease with high morbidity, disability, and mortality, which seriously threatens the health and life of middle-aged and elderly people [1], and it is becoming a serious medical and social concern. According to WHO statistics, the global prevalence of cerebrovascular disease is (500–600)/10<sup>5</sup>, and the prevalence rate in China is 720/10<sup>5</sup>. Among them, ischemic cerebrovascular diseases account for 70–80% [2]. Many neuroprotective drugs have been developed, designed to reduce excitatory amino acid toxicity, lessen intracellular calcium overload, inhibit neuronal inflammation and apoptosis, and improve mitochondrial function [3,4]. However, the clinical efficacy of currently available neuroprotective drugs is limited, so further research is needed.

During cerebral ischemia, GLU is released in large quantities and accumulates in the synaptic cleft, followed by overwhelmed activation of GLU receptors, resulting in neuronal necrosis and apoptosis, which is called GLU excitotoxicity [5]. Excessive GLU can over-activate the NMDA receptors of neurons and cause oxidative stress and calcium overload [6,7]. Previous studies [8,9] have shown that GLU can lead to decreased MMP and regulate the expression of bcl-2 family proteins through modulating multiple apoptotic signals to induce apoptosis.

Dexmedetomidine (DEX) is a new type of highly selective, highly specific  $\alpha$  2-adrenergic agonist with the effects of sedation, analgesia, and anti-sympathetic activity [10,11]. Its unique pharmacological characteristics make it a new type of anesthesia adjuvant drug widely used in intensive care and clinical anesthesia. A wide array of studies have reported that the protective effects of DEX on ischemic cerebral impairment involve multiple possible mechanisms [12–14], including inhibition of catecholamine release, reduction of neurotoxicity of excitatory amino acids, decreased neuronal apoptosis, and anti-neuroinflammatory activity. However, the specific mechanism of DEX remains unclear.

Thus, in this study, PC12 cells were treated with high-concentration GLU to create a model of neuronal hypoxic injury. We sought to investigate the protective effect of DEX and explore the underlying mechanism in regulation of GLU-induced neurotoxicity in PC12 cells.

## Material and Methods

### Cell culture

The protocols for experimentation described in this article were approved by the Animal Care Committee of the Chinese People's Liberation Army General Hospital. PC12 cells derived

from pheochromocytoma of the rat adrenal medulla were donated by Professor Hu Gang, Nanjing Medical University. After thawing, cells were plated on culture flasks and kept in DMEM containing 10% fetal bovine serum and 10<sup>5</sup> U/L penicillin and 100 mg/L streptomycin at 37°C in a humidified atmosphere of 5% CO<sub>2</sub>. Cells were sub-cultured once every 3 days, and only those in the exponential growth phase were utilized in experiments. Cells were seeded in 6-well plates at a density of 5×10<sup>5</sup>/ml (2 ml per well) or in a 96-well plate at a density of 1×10<sup>5</sup>/ml (100 ul per well) and subsequently incubated for 24 h.

### Experimental protocols

DEX was dissolved in normal saline. The cells were randomly assigned into 4 groups. In the Control (Con) group, the cells were pretreated with normal saline for 1 h and then incubated in serum-free DMEM for 24 h. In the DEX group, the cells were pretreated with 1  $\mu$ M DEX for 1 h and then incubated in serum-free DMEM for 24 h. In the GLU group, the cells were pretreated with normal saline for 1 h and then incubated with 20 mM GLU for 24 h. In the DEX+GLU group, the cells were pretreated with 1  $\mu$ M DEX for 1 h and then incubated with 20 mM GLU for 24 h. Cells in each group were collected and centrifuged at 2000 rpm for 5 min. After discarding the supernatant, the cells were lysed with 1% Triton (200  $\mu$ L) in an ice bath for 20 min and centrifuged at 4°C at 12 000 g for 10 min. Then, the supernatant was collected for assessment.

In the preliminary experiments, we treated PC12 cells with GLU at a concentration of 20 mM for 24 h and found that they became smaller and irregular in shape under the microscope. In addition, the concentration of GLU in many PC12 cell injury models was also 20 mM [15,16]. Therefore, we chose this concentration as a cell damage model for subsequent experiments.

### Reagents and antibody

Cell culture supplies and media were purchased from Gibco, fetal bovine serum was from MP Biomedicals, trypsin, MTT Cell Proliferation and Cytotoxicity Assay Kit was from Amresco, and penicillin-streptomycin was from Hua Yao Southern Pharmaceutical Co. (Shenzhen, China). DEX was purchased from Aladdin Biochemical Science and Technology Co. (Shanghai, China). GLU was purchased from Sinopharm Group Chemical Reagent Co. Cellular malondialdehyde (MDA) test kits and superoxide dismutase (SOD) assay kits were purchased from the Bioengineering Institute (Nanjing, China). Reactive oxygen species (ROS) assay kit, mitochondrial membrane potential assay kit, and Annexin V-FITC apoptosis assay kit were purchased from Beyotime Biotechnology Co. (Shanghai, China). Fluo-8<sup>®</sup> Calcium Reagents and Screen Quest™ Fluo-8 NW calcium assay kits were purchased from AAT BioQuest (USA). BCA Protein Quantification Kits were purchased from Thermo Pierce

(Rockford, IL, USA). Rabbit anti-mouse Caspase-3, Caspase-9, Bax, and Bcl-2 IgG antibodies and goat anti-rabbit HRP were purchased from Abcam (UK).

### Determination of cell viability

PC12 cells were seeded at a density of  $1 \times 10^5$ /ml in a 96-well plate (100  $\mu$ l per well) at 37°C with 5% CO<sub>2</sub>. Supernatant was replaced with serum-free DMEM medium after 24-h culture, followed by another 24-h incubation. We added 10  $\mu$ l free DMEM medium containing MTT (5 mg/ml), followed by 4-h incubation. We removed the culture medium, then added 100  $\mu$ l 2-methyl sulfoxide to each well. The absorbance at 570 nm wavelength was measured with an enzyme scale (Molecular Devices, USA) after the blue violet crystals completely dissolved.

### Determination of apoptosis rate

PC12 cells were seeded at a density of  $5 \times 10^5$ /ml in a 6-well plate (2 ml per well) at 37°C with 5% CO<sub>2</sub> for 24 h. Cells were collected in the same manner as described above in experimental protocols. Apoptosis rates were analyzed using the Annexin V-FITC apoptosis assay kit. Briefly, cells were collected according to the manufacturer's instructions and resuspended with 195  $\mu$ l annexin V-FITC binding solution in EP tubes. Subsequently, Annexin V-FITC (5  $\mu$ l) and propidium iodide staining solution (10  $\mu$ l) were added into it and incubated at room temperature for 20 min and then subjected to ice bath for 10 min. The FSC VS SSC scatter plot was used to delineate all cells (at least 20000 cells from each sample) except adhesion and cell fragments, and then the 2 fluorescence parameters of the cells in the gate were analyzed with the Annexin V VS PI dot plot to obtain the proportion of early apoptotic and necrotic cells.

### Malondialdehyde (MDA) content

Cells were collected (the same as Experimental protocols above) after 24h culture at a density of  $5 \times 10^5$ /ml in a 6-well plate and mixed with an extract of 300  $\mu$ l reagent V for 2 min. Then 0.1 mL was sampled into a 1.5 ml EP tube. After adding other reagents according to the instructions of the kit, 0.25 ml of the solution was pipetted into a 96-well plate, and the 530nm wavelength OD value was read by the Microplate Reader (Molecular Devices, USA). MDA content was calculated according to the formula of the commercial assay kits.

### Superoxide dismutase (SOD) activity

Cells collection is the same as Experimental protocols above. Reagents were added according to the instructions of the assay kits and the 450 nm wavelength OD value was read out by the Microplate Reader. SOD activity was obtained according to the formula of the kits.

### Reactive oxygen species (ROS) content

DCFH-DA was diluted with serum-free medium at 1: 1000 to a final concentration of 10  $\mu$ M. Cells were collected (the same as Experimental protocols above) and suspended in diluted DCFH-DA and incubated at 37°C for 20 min. Mixed it up every 3-5 min to make the probe fully contact with the cells. Cells were washed 3 times with serum-free culture medium to fully remove DCFH-DA that has not entered the cells. Cells were collected and analyzed (at least 20000 cells from each sample) by flow cytometry. The mean fluorescence intensity (MFI) in each group was measured by setting an excitation wavelength of 488 nm and an emission wavelength of 530 nm.

### Ca<sup>2+</sup> content in cells

Fluo-8 was dissolved in DMSO to prepare a Fluo-8 stock solution at a concentration of 2-5 mM. The stock solution was diluted with PBS buffer to a Fluo-8 working solution at a concentration of 4-5  $\mu$ M. Added equal volumes of Fluo-8 working solution to the collected cell suspension (1 ml). Incubated for half an hour in a 37°C incubator. Centrifuged and discarded the supernatant then washed the cells 3 times with PBS. Cell suspensions were collected (at least 20000 cells from each sample), stained with Fluo-8, and detected by flow cytometry. Flow cytometric data was analyzed using CellQuest™ Pro software.

### Mitochondrial membrane potential measurement

$10^5$  cells were collected and resuspended in 0.5 ml cell culture medium. Added 0.5 ml JC-1 staining solution, mixed well then incubated at 37°C for 20 min. Centrifuged at 600 g for 4 min at 4°C and discarded the supernatant. The cells were resuspended in 1 ml JC-1 staining buffer (1X), and centrifuged at 600 g, 4°C for 3-4 min. Supernatant was discarded, repeated once. Resuspended the cell pellets again with JC-1 staining buffer (1X) and detected (at least 20000 cells from each sample) by flow cytometry. The MFI of red and green in each group was measured by setting an excitation wavelength of 525 nm and an emission wavelength of 595 nm. The ratio of red-green fluorescence represented the level of mitochondrial membrane potential.

### Western blot analysis

Cells were seeded in 6-well plates at a density of  $5 \times 10^5$ /ml (2 ml per well). 24h after incubation and 24 h after experimental treatment, cells were washed 2-3 times with PBS then lysed in protein extraction working solution for 3-5 minutes with shaking. Scraped and lysis was collected in 1.5 ml centrifuge tube. Incubated in for 30 min, repeatedly pipetting several times every 10 minutes to ensure complete cell lysis. Centrifuged at 12000g for 5 min then collected the supernatant.

BCA method was used to quantify protein according manufacturer's instruction, then added loading buffer and boiled for 5 min at 100°C. Proteins were loaded onto SDS-PAGE gel and transferred to PVDF membrane. 5% BSA was added to block at room temperature for 2 h, Incubated with the primary antibody overnight at 4°C on a horizontal shaker. Membrane was washed 3 times for 5 min each time. 5% BSA-TBST was added to dilute secondary antibody and incubate for 40 min at room temperature. Then, the membrane was incubated with ECL solution and exposed.

### Statistical analysis

All data processing was analyzed by a non-participant in the experiment. Data were expressed as the mean±standard error (SE). Intergroup comparisons were conducted by two-way ANOVA followed by Tukey's post hoc test to determine significant differences between the experimental groups. *P* values <0.05 were considered statistically significant.

## Results

### Effect of DEX on cell viability and apoptosis in GLU-injured PC12 cells

Pilot experiments showed that GLU at 20 mM had a moderate damage to cells [15,16], thus our cell hypoxia injury model is based on this concentration. In preliminary experiments, cells were pretreated with different concentrations of DEX (0.01–100 μmol/l) for 1 h, then GLU was added. DEX at 0.01 μM showed a protective effect, and the higher the concentration, the stronger the protective effect. While 1 μM DEX had a cytoprotective effect in many other studies [17,18]. So, in this experiment, we chose 1 μM as the moderate concentration of DEX.

Cell viability was increased to (82.3±4.0)% of Con group, and the difference was statistically significant compared with GLU group (*P* <0.05), while DEX had no effect on cell viability (Figure 1A). Consistently, DEX has no effect on apoptosis rate (Figure 1B, *P*>0.05), while the apoptosis rate of DEX+GLU group is significantly lower than that of GLU group (Figure 1B, *P*<0.05).

### Effect of DEX on oxidative stress in GLU-damaged PC12 cells

Figure 2A showed that the MDA content in GLU group increased to (2.6±0.1) nmol/mg (compared with the Con group, *P*<0.05), interestingly, it decreased to (2.0±0.1) nmol/mg with DEX pretreatment (compared with GLU group, *P*<0.05). In Figure 2B, SOD activity of GLU group decreased to (19.2±0.8) U/mg, while DEX increased the SOD activity to (22.7±0.6) U/mg, which was significantly higher than that of GLU group (*P*<0.05). The mean

fluorescence intensity (MFI) of DCF reflects the level of intracellular reactive oxygen species. Figure 2C showed that compared with Con group, the MFI of GLU group increased from 170.1±14.4 to 516.7±19.0 (*P*<0.05), and the MFI decreased to 310.4±19.5 after DEX pretreatment, which was significantly lower than that of the GLU group (*P*<0.05).

### Effect of DEX on calcium homeostasis in GLU-damaged PC12 cells

Since intracellular calcium overload is responsible for cell necrosis and apoptosis [19], we further explored the potential role of DEX in regulating calcium homeostasis. The average fluorescence intensity of Fluo 8 was used to reflect intracellular calcium level. Figure 3 showed that the MFI of GLU group increased from 103.1±7.8 (Con group) to 160.0±8.0 (*P*<0.05). DEX pretreatment reduced the MFI to 129.9±5.6, which was significantly lower than that of GLU group (*P*<0.05).

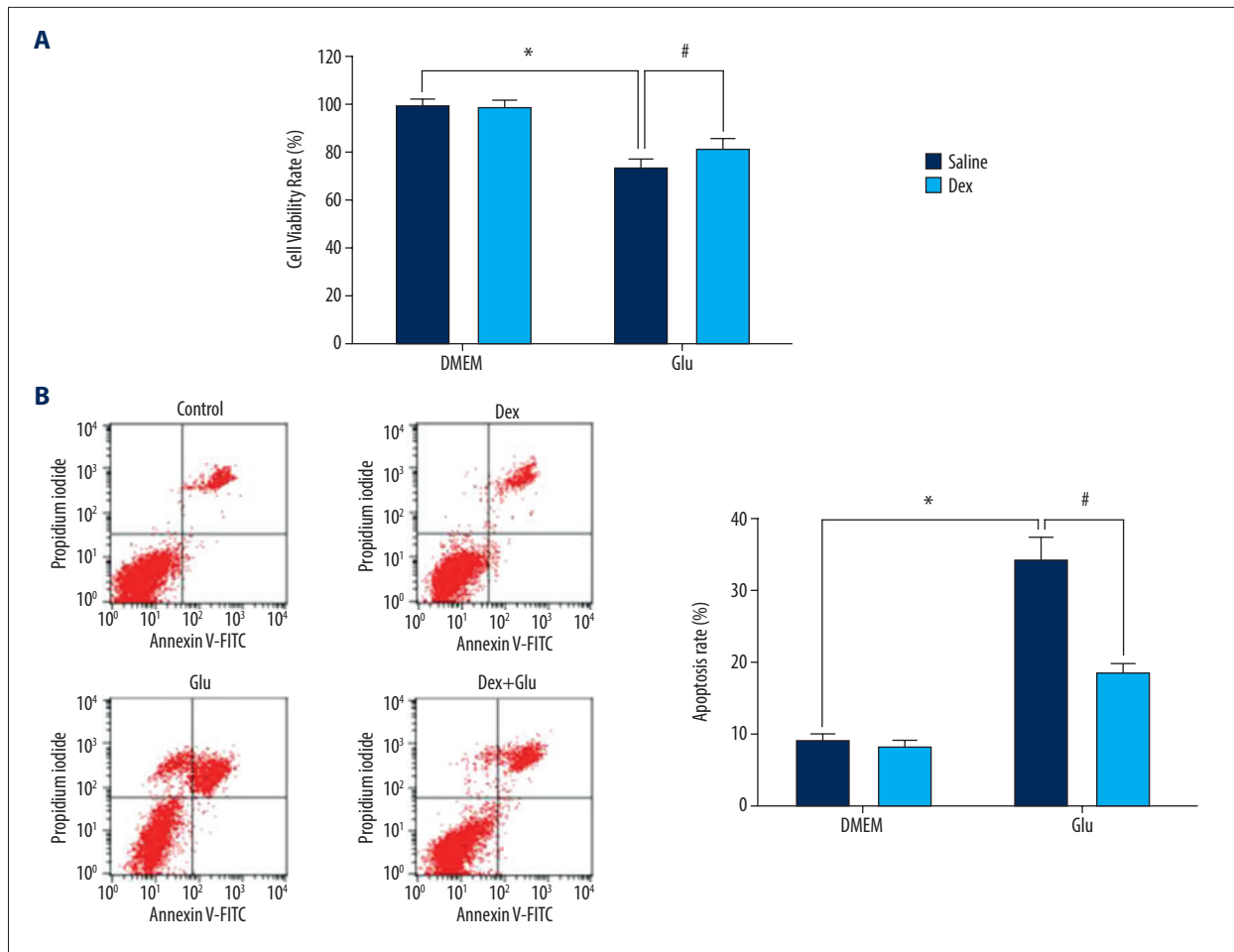
### Effect of DEX on mitochondria-mediated apoptosis pathway in GLU-damaged PC12 cells

Mitochondria-mediated apoptotic pathway is one of the main pathways of apoptosis [20], in order to further explore its mechanism, we explored the effect of DEX on mitochondrial membrane potential of GLU-injured PC12 cells. Changes in mitochondrial membrane potential was detected after JC-1 staining, Figure 4 showed that the MFI ratio of GLU group decreased from 3.4±0.1 to 2.2±0.2 (*P*<0.05) compared with the Con group. Compared with the GLU group, DEX increased the MFI to 2.9±0.2, which was significantly higher than that of GLU group (*P*<0.05).

### Effect of DEX on the expression of mitochondrial apoptosis-related proteins in GLU- injured PC12 cells

We further explored its effect on apoptosis-related proteins. Caspase-3 is in the downstream of the apoptotic pathway and plays an important role in the process of apoptosis [21]. Expression of caspase-3 was significantly increased after GLU treatment on PC12 cells (Figure 5A, *P*<0.05). In DEX group, the expression of caspase-3 protein was significantly decreased (*P*<0.05).

Caspase-9 is an upstream molecule of apoptosis pathway and it binds to cytochrome c and Apaf-1, which is released by mitochondria, to form a complex to induce apoptosis. It is a marker of mitochondria-mediated apoptosis pathway [22]. We found that GLU significantly increased the expression of caspase-9 (Figure 5B, *P*<0.05) while its expression was significantly decreased after DEX pretreatment (*P*<0.05).



**Figure 1.** Effect of DEX on cell viability rate (A) and apoptosis rate (B) in GLU-injured PC12 cells. The data are represented as mean±SE. n=6. \*  $p < 0.05$  vs. Con group, #  $p < 0.05$  vs. GLU group.

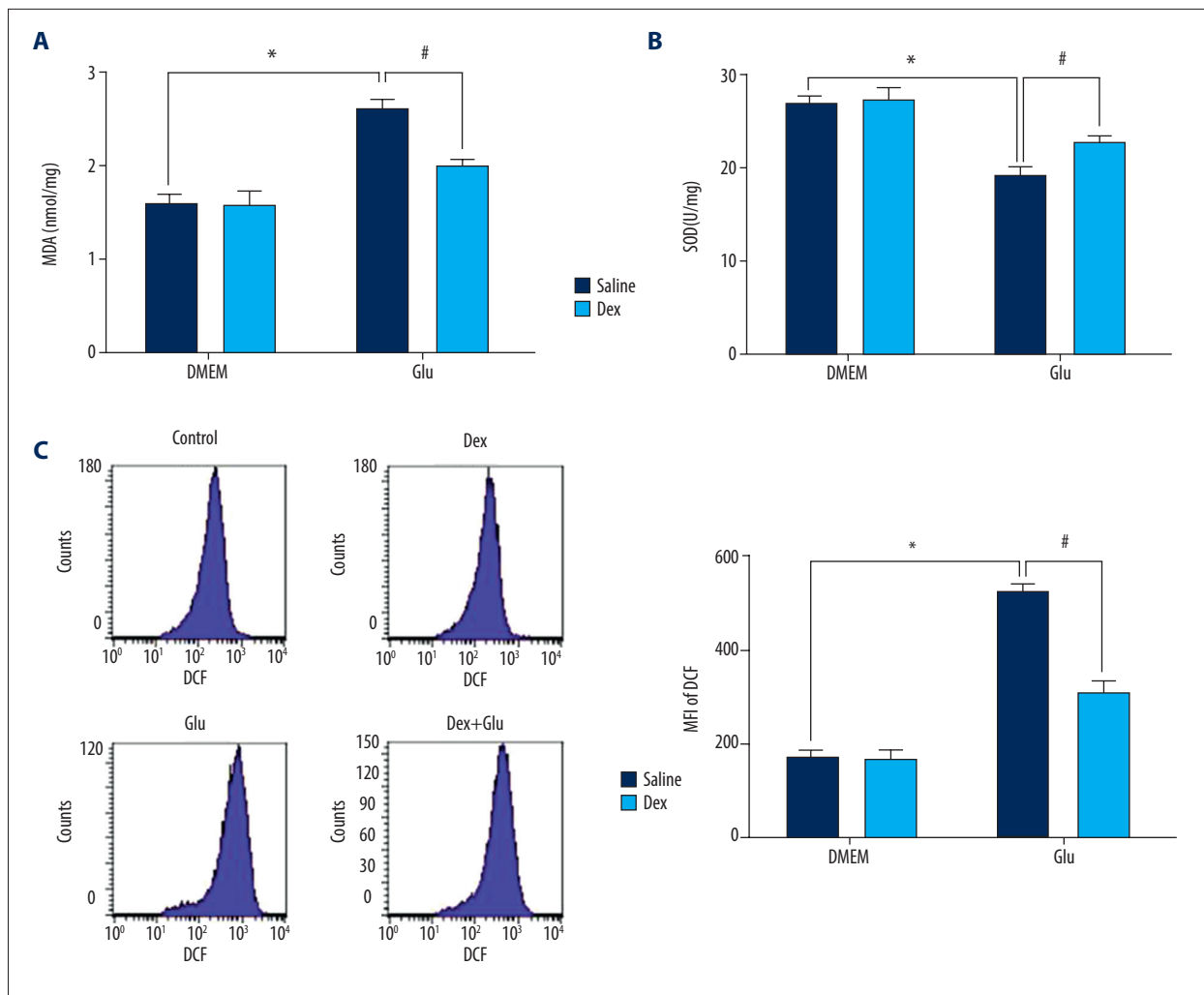
The release of Cytochrome C (Cyt-C) from mitochondria stimulated by apoptotic signals is critical in mitochondrial-mediated apoptosis. As shown in Figure 5C, the expression of Cyt-C protein increased significantly when we added GLU (Figure 5C,  $P < 0.05$ ); consistently, the expression of Cyt-C protein decreased significantly with DEX pretreatment (Figure 5C,  $P < 0.05$ ).

Bax and Bcl-2 are both important members of the bcl-2 family. Bax can promote apoptosis and it increases mitochondrial membrane permeability by activating pro-apoptotic proteins, thus affecting mitochondrial membrane potential, resulting in apoptotic protein release and apoptosis [23,24]. Bcl-2 is an apoptosis-inhibiting protein which can inhibit mitochondrial release of pro-apoptotic factors [25–27]. Thus, we further detected Bax and Bcl-2 expression and found that GLU significantly increased the expression of bax protein in PC12 cells (Figure 5D,  $P < 0.05$ ). DEX significantly downregulated the expression of bax protein (Figure 5D,  $P < 0.05$ ), indicating a possible mechanism by which DEX may regulate GLU-induced apoptosis by affecting Bcl-2 expression (Figure 5E,  $P < 0.05$ ) and downstream apoptosis signals.

## Discussion

We found that GLU significantly reduced cell viability and increased apoptosis rate, which was ameliorated by pretreatment with DEX. The protective effect of DEX on GLU-induced cytotoxicity in PC12 cells may be related to its anti-oxidant activity and reduction of intracellular calcium overload, as well as its ability to inhibit the mitochondria-mediated apoptotic pathway.

Excessive GLU is shown to be involved in neuronal cell death and apoptosis in a number of ischemic cerebrovascular diseases [8,9]. In the present study, PC12 cells were used to represent a model of neurons. PC12 cells, a common cell line derived from adrenal medullary pheochromocytoma of rats, have typical structural and functional characteristics of neuroendocrine cells, and they may replace primary cultured neurons in neurophysiological and pharmacological research [28]. In the preliminary experiment, PC12 cells treated with 20 mM GLU exhibited reduced cell viability and increased apoptosis rate. These findings indicate it could be used as a model to mimic



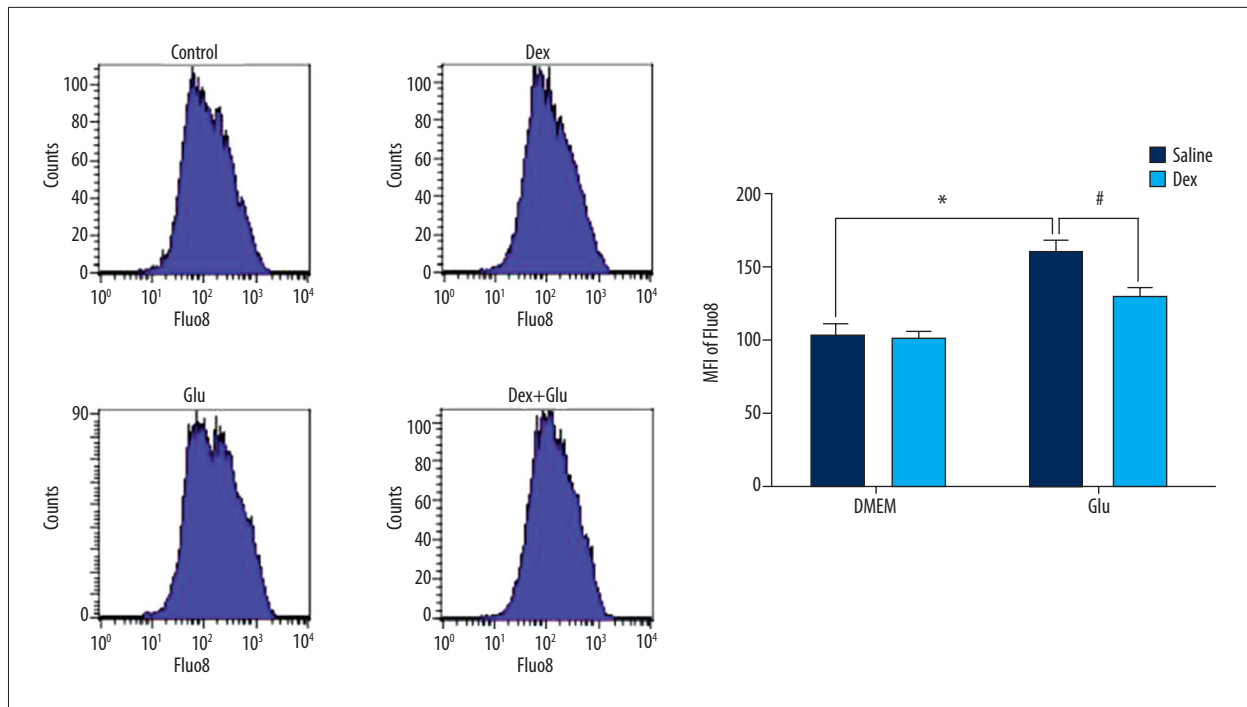
**Figure 2.** Effect of DEX on MDA content (A), SOD activity (B), and ROS level (C) in GLU-exposed PC12 cells. The data are represented as mean±SE. n=6. \* p<0.05 vs. Con group; # p<0.05 vs. GLU group

the pathological process of ischemic cerebrovascular disease *in vitro*, as reported in previous reports [8,9,29].

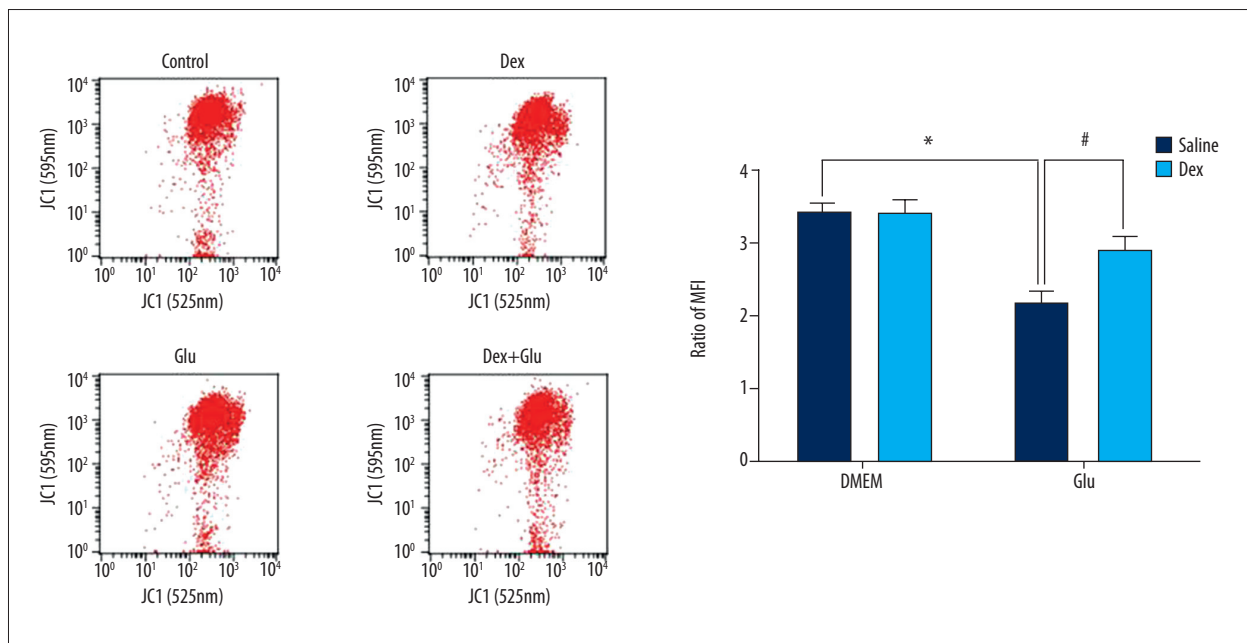
DEX is a potent and highly specific  $\alpha_2$ -adrenergic agonist that is widely used in clinical practice as an auxiliary anesthetic [30]. Recently, many studies [31–33] reported that DEX has a certain neuroprotective effect, which has attracted widespread attention. However, there is no evidence on whether DEX provides a neuroprotective effect against GLU-induced neurotoxicity in PC12 cells. Our study used a GLU-induced model to investigate the neuroprotective effect of DEX *in vitro* and its pertinent action mechanisms. Based on the preliminary study [17,18], PC12 cells were treated with 1  $\mu$ M DEX in the present study. Obvious increases in viability and reduction in apoptosis rate were observed in the cells pretreated with DEX, which indicates that DEX exerts neuroprotective effects against GLU-induced neurotoxicity in PC12 cells. Previous *in vitro* studies [34,35] have shown that the DEX attenuates

propofol-induced neurotoxicity in primary-cultured neurons, which is consistent with the present study, indicating that DEX can exert neuroprotective effects in different experimental models. Our study also investigated whether anti-oxidant property and inhibition of calcium overload and apoptosis are involved in the neuroprotective effects of DEX.

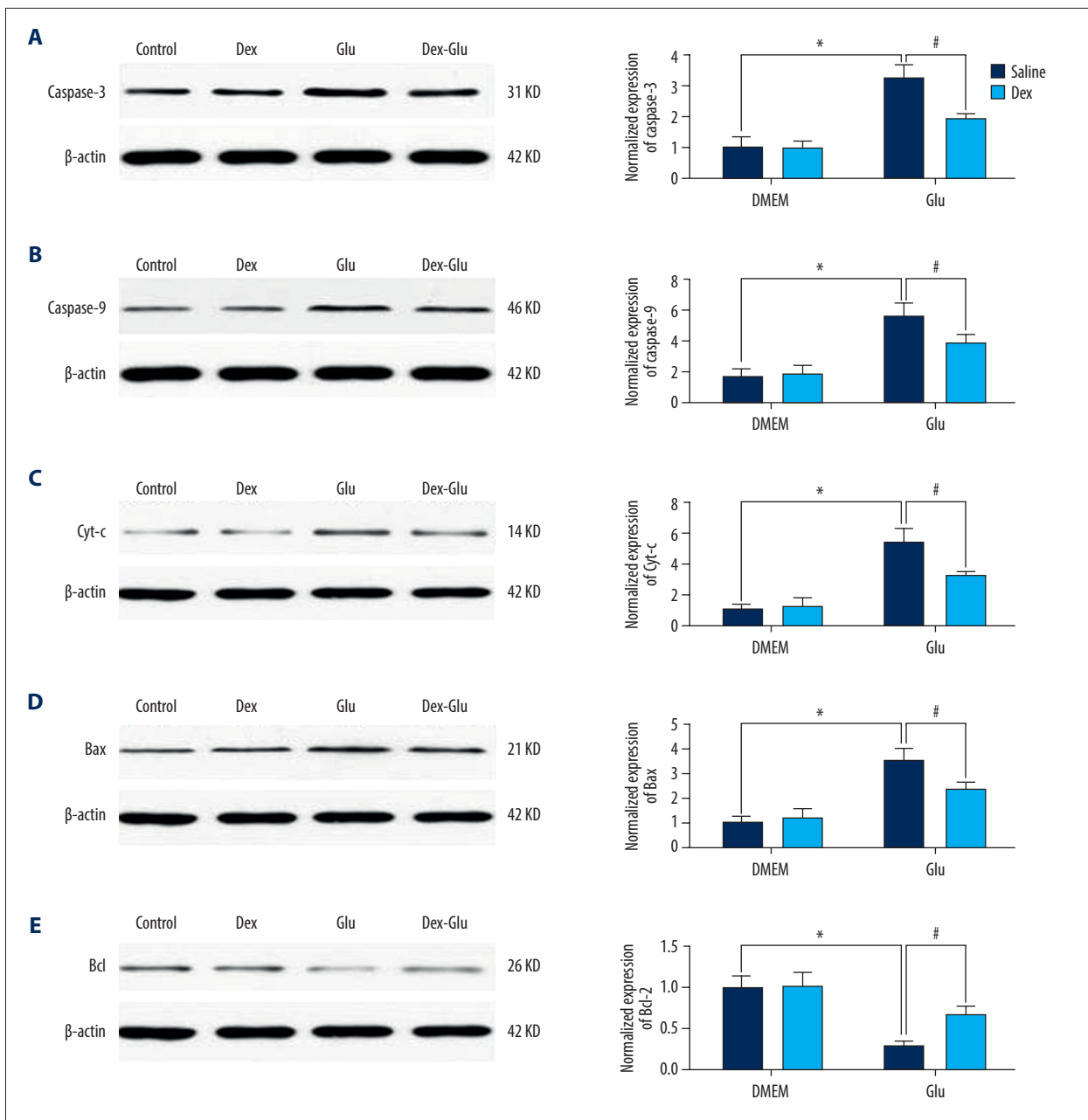
During cerebral ischemia, excessive GLU inhibits the function of glutamate/cystine transporters and reduces the intake of cystine, which leads to reduced synthesis of glutathione and oxidative stress [15]. Oxidative stress involves accumulation of reactive oxygen species (ROS), which harms cellular function and structure and ultimately causes necrosis and apoptosis [36]. SOD is an important free radical scavenger, whose function partly reflects the body's ability to eradicate oxygen free radicals [37]. MDA, SOD, and ROS are common indicators used to evaluate the extent of oxidative damage. In our study, administration of DEX significantly decreased MDA and ROS



**Figure 3.** Effect of DEX on level of intracellular  $Ca^{2+}$  by MFI of Fluo8 in GLU-induced PC12 cells. The data are represented as mean $\pm$ SE of 3 independent experiments. \*  $p < 0.05$  vs. Con group, #  $p < 0.05$  vs. GLU group.



**Figure 4.** Effect of DEX on mitochondrial membrane potential (MMP) by ratio of MFI of J-aggregates to JC-1 in GLU-induced PC12 cells. The data are represented as mean $\pm$ SE, n=6. \*  $p < 0.05$  vs. Con group, #  $p < 0.05$  vs. GLU group.



**Figure 5.** Effect of DEX on the protein expression of caspase-3 (A), caspase-9 (B), cyt-c (C), bax (D), and bcl-2 (E) in GLU-injured PC12 cells. The data are represented as mean±SE of 3 independent experiments. \*  $p < 0.05$  vs. Con group, #  $p < 0.05$  vs. GLU group.

levels and increased SOD activity, suggesting that DEX inhibits oxidative stress induced by GLU. These results are partially consistent with a previous study [38], in which DEX was shown to alleviate injury to primary cultured neurons from rats induced by glucose deprivation through reducing intracellular ROS levels and inhibiting oxidative stress. Eser et al. [39] also found that DEX exerts neuroprotective effects by reducing MDA and NO levels and increasing SOD activity in rats with transient global cerebral ischemia-reperfusion injury. Based on the above results, we speculate that this anti-oxidant property is one of

the main mechanisms by which DEX protects PC12 cells from GLU-induced neurotoxicity.

Calcium overload is recognized as an important mechanism in the pathological process of cerebral ischemia and hypoxia injury [40]. Studies [41,42] have proved GLU can mediate the opening of calcium channels and cause intracellular calcium overload through over-activation of certain receptors such as the NMDA receptors in nerve cells. This may be related to the activation of a variety of calcium-dependent proteases in the



cell, which leads to structural damage and functional metabolic disorders [43]. Previous research [44] demonstrated that DEX attenuated oxidative stress, downregulated NOX2 protein expression, and inhibited intracellular calcium overload in CoCl<sub>2</sub>-treated PC12 cells. Another study [45] found that DEX pretreatment protected cardiomyocytes against oxygen-glucose deprivation/reoxygenation-induced damage by inhibiting calcium overload and apoptosis. In this study, DEX significantly decreased Ca<sup>2+</sup> levels induced by GLU, indicating that DEX protects PC12 cells from damage by inhibiting calcium overload, which provides a new possible action mechanism for further clarifying the neuroprotective effect of DEX.

Stabilization of MMP is indispensable to maintain normal physiological function of mitochondria. Oxidative stress and calcium overload can result in decreased MMP and subsequent release of cytochrome c, which activates cascade reaction of caspases and ultimately leads to apoptosis [46,47]. Due to its precedence to mitochondrial pathological changes, MMP is a sensitive indicator for evaluating mitochondrial function. Studies [48] have shown that apoptosis can be inhibited by preventing the decline of mitochondrial membrane potential. Li et al. [49] proved that DEX can suppress apoptosis in hippocampal neurons of neonatal rats induced by isoflurane by activating the PI3K/Akt anti-apoptotic pathway. In this study, DEX mitigated the reduction of MMP in PC12 cells caused by GLU and decreased the apoptosis rate, suggesting that the neuroprotective effect of DEX is related to the upregulation of MMP.

It is well known that the mitochondria-mediated apoptosis pathway is one of the main pathways of apoptosis. Bcl-2 family proteins play a key role in regulating apoptosis [50]. The activation of pro-apoptotic bax can trigger decrease of MMP and release of cyt-c, which activates caspase-9 and downstream

caspase-3 by cascade reaction to induce apoptosis, while the anti-apoptotic bcl-2 acts in the opposite way. Caspase-9 is in upstream of the apoptotic pathway and can further activate downstream caspases [51]. The downstream caspase-3 is the main executor of apoptosis and is a common node of various apoptotic pathways [52]. Sanders et al. [53] confirmed that DEX inhibits apoptosis of cortical neurons in both *in vivo* and *in vitro* models by upregulating the expression of anti-apoptotic proteins Bcl-2 and pERK and inhibiting the expression of caspase-3. Jeon et al. [54] suggested that DEX pretreatment inhibits apoptosis through upregulating the expression of anti-apoptotic protein bcl-2 and downregulating the expression of pro-apoptotic protein bax. In this study, DEX pretreatment significantly decreased the expression levels of bax, cyt-c, caspase-9, and caspase-3 and increased the expression level of Bcl-2. These findings revealed that the protective effect of DEX against GLU-induced neuronal damage was associated with its inhibition of the mitochondria-mediated apoptosis pathway.

## Conclusions

In conclusion, the present study showed that dexmedetomidine attenuated GLU-induced neurotoxicity in PC12 cells, and this may be attributed to its anti-oxidant property, reduction of intracellular calcium overload, and inhibition of the mitochondria-mediated apoptotic pathway. These findings provide new insights into the mechanism of DEX and indicate its potential application for the treatment of ischemic cerebrovascular disease.

## Conflict of interest

None.

## References:

1. Del Bene A, Palumbo V, Lamassa M et al: Progressive lacunar stroke: Review of mechanisms, prognostic features, and putative treatments. *Int J Stroke*, 2012; 7: 321–29
2. Zhou M, Offer A, Yang G et al: Body mass index, blood pressure, and mortality from stroke: A nationally representative prospective study of 212,000 Chinese men. *Stroke*, 2008; 39: 753–59
3. Christophe BR, Mehta SH, AL Garton: Current and future perspectives on the treatment of cerebral ischemia. *Expert Opin Pharmacother*, 2017; 18: 573–80
4. Sifringer M, von Haefen C, Krain M et al: Neuroprotective effect of dexmedetomidine on hyperoxia-induced toxicity in the neonatal rat brain. *Oxid Med Cell Longev*, 2015; 2015: 530371
5. Peng M, Ling X, Song R et al: Upregulation of GLT-1 via PI3K/Akt Pathway Contributes to Neuroprotection Induced by Dexmedetomidine. *Front Neurol*, 2019; 10: 1041
6. Park CH, Song JH, Kim SN et al: Neuroprotective effects of tetrahydrocurcumin against glutamate-induced oxidative stress in hippocampal HT22 cells. *Molecules (Basel, Switzerland)*, 2019; 25: 10
7. Angelova PR, Vinogradova D, Neganova ME et al: Pharmacological sequestration of mitochondrial calcium uptake protects neurons against glutamate excitotoxicity. *Mol Neurobiol*, 2019; 56: 2244–55
8. Kim JJ, Kang YJ, Shin SA et al: Phlorofucofuroeckol improves glutamate-induced neurotoxicity through modulation of oxidative stress-mediated mitochondrial dysfunction in PC12 cells. *PLoS One*, 2016; 11: e0163433
9. AM Sabogal-Guáqueta, F Hobbie, A Keerthi et al: Linalool attenuates oxidative stress and mitochondrial dysfunction mediated by glutamate and NMDA toxicity. *Biomed Pharmacother*, 2019; 118: 109295
10. Carollo DS, Nossaman BD, Ramadhyan U: Dexmedetomidine: A review of clinical applications. *Curr Opin Anaesthesiol*, 2008; 21: 457–61
11. KM Chiu, TY Lin, MY Lee et al: Dexmedetomidine protects neurons from kainic acid-induced excitotoxicity by activating BDNF signaling. *Neurochem Int*, 2019; 129: 104493
12. Mian P, Yan-Lin W, Cheng-Yao W et al: Dexmedetomidine attenuates lipopolysaccharide-induced proinflammatory response in primary microglia. *J Surg Res*, 2013; 179: e219–25
13. Chiu KM, Lin TY, Lu CW, Wang SJ: Inhibitory effect of glutamate release from rat cerebrocortical nerve terminals by  $\alpha 2$  adrenoceptor agonist dexmedetomidine. *Eur J Pharmacol*, 2011; 670: 137–47
14. Li J, Xiong M, Nadavaluru PR et al: Dexmedetomidine attenuates neurotoxicity induced by prenatal propofol exposure. *J Neurosurg Anesthesiol*, 2015; 28: 51

15. Chang Ch, Chen KC, Liaw KC et al: Astaxanthin protects PC12 cells against homocysteine- and glutamate-induced neurotoxicity. *Molecules* (Basel, Switzerland), 2020; 25: 17
16. Liu Y, Liu L, Ying XX et al: Dried Rehmannia root protects against glutamate-induced cytotoxicity to PC12 cells through energy metabolism-related pathways. *Neural Regen Res*, 2017; 12: 1338–46
17. Wang Y, Mao X, Chen H et al: Dexmedetomidine alleviates LPS-induced apoptosis and inflammation in macrophages by eliminating damaged mitochondria via PINK1 mediated mitophagy. *Int Immunopharmacol*, 2019; 73: 471–81
18. Schoeler M, Loetscher PD, Rossaint R et al: Dexmedetomidine is neuroprotective in an *in vitro* model for traumatic brain injury. *BMC Neurol*, 2012; 12: 20
19. Orrenius S, Zhivotovsky B, Nicotera P: Regulation of cell death: The calcium-apoptosis link. *Nature reviews Mol Cell Biol*, 2003; 4: 552–65
20. Ling K, Mingzhao D, Chaupu Z et al: Polysaccharide purified from Lycium barbarum protects differentiated PC12 cells against L-Glu-induced toxicity via the mitochondria-associated pathway. *Mol Med Rep*, 2017; 16: 5533–40
21. Porter AG, Jänick RU: Emerging roles of caspase-3 in apoptosis. *Cell Death Differ*, 1999; 6: 99–104
22. Riedl SJ, Salvesen GS: The apoptosome: Signalling platform of cell death. *Nat Rev Mol Cell Biol*, 2007; 8: 405–13
23. Green DR, Reed JC: Mitochondria and apoptosis. *Sciences*, 1998; 281: 1309–12
24. Garner TP, Amgalan D, Reyna DE et al: Small-molecule allosteric inhibitors of BAX. *Nat Chem Biol*, 2019; 15: 322–30
25. Kale J, Osterlund EJ, Andrews DW: BCL-2 family proteins: changing partners in the dance towards death. *Cell Death Differ*, 2018; 25: 65–80
26. Czabotar PE, Lessene G, Strasser A et al: Control of apoptosis by the BCL-2 protein family: Implications for physiology and therapy. *Nat Rev Mol Cell Biol*, 2013; 15: 49–63
27. Jeong JK, Lee JH, Moon JH et al: Melatonin-mediated  $\beta$ -catenin activation protects neuron cells against prion protein-induced neurotoxicity. *J Pineal Res*, 2014; 57: 427–34
28. Yu W, Deng X, Ma Z et al: Equol protects PC12 neuronal cells against hypoxia/reoxygenation injury *in vitro* by reducing reactive oxygen species production. *Nan Fang Yi Ke Da Xue Xue Bao*, 2016; 36(1): 1–7
29. Kim JY, Kim HJ, Kim N et al: Effects of radiofrequency field exposure on glutamate-induced oxidative stress in mouse hippocampal HT22 cells. *Int J Radiat Biol*, 2017; 93: 249–56
30. Kim JC, Kim J, Kwak H, Ahn SW: Premedication with dexmedetomidine to reduce emergence agitation: a randomized controlled trial. *BMC Anesthesiol*, 2019; 19: 144
31. Chi OZ, Hunter C, Liu X et al: The effects of dexmedetomidine on regional cerebral blood flow and oxygen consumption during severe hemorrhagic hypotension in rats. *Anesth Analg*, 2011; 113: 349–55
32. Chi OZ, Grayson J, Barsoum S et al: Effects of dexmedetomidine on microregional O<sub>2</sub> balance during reperfusion after focal cerebral ischemia. *J Stroke Cerebrovasc Dis*, 2015; 24: 163–70
33. Degos V, Charpentier TL, Chhor VC et al: Neuroprotective effects of dexmedetomidine against glutamate agonist-induced neuronal cell death are related to increased astrocyte brain-derived neurotrophic factor expression. *Anesthesiology*, 2013; 118: 1123–32
34. Wei Y, Hu J, Liang Y et al: Dexmedetomidine pretreatment attenuates propofol-induced neurotoxicity in neuronal cultures from the rat hippocampus. *Mol Med Rep*, 2016; 14: 3413
35. Tu Y, Liang Y, Xiao Y et al: Dexmedetomidine attenuates the neurotoxicity of propofol toward primary hippocampal neurons *in vitro* via Erk1/2/CREB/BDNF signaling pathways. *Drug Des Devel Ther*, 2019; 13: 695–706
36. Hu XB, Xi ZY, Liu LQ et al: Dexmedetomidine promotes SH-SY5Y cell resistance against impairment of iron overload by inhibiting NF- $\kappa$ B pathways. *Neurochem Res*, 2019; 44: 959–67
37. Li Y, Liu S: The effect of dexmedetomidine on oxidative stress response following cerebral ischemia-reperfusion in rats and the expression of intracellular adhesion molecule-1 (ICAM-1) and S100B. *Med Sci Monit*, 2017; 23: 867–73
38. Rodríguez-González R, Sobrinho T, Veiga S et al: Neuroprotective effects of dexmedetomidine conditioning strategies: Evidences from an *in vitro* model of cerebral ischemia. *Life Sci*, 2015; 144: 162–69
39. Eser O, Fidan H, Sahin O et al: The influence of dexmedetomidine on ischemic rat hippocampus. *Brain Res*, 2008; 1218: 250–56
40. Lee J-S, Kim W-Y, Jeon Y-J et al: Aquilariae Lignum extract attenuates glutamate-induced neuroexcitotoxicity in HT22 hippocampal cells. *Biomed Pharmacother*, 2018; 106: 1031–38
41. Maiolino M, O'Neill N, Lariccia V et al: Inorganic polyphosphate regulates AMPA and NMDA receptors and protects against glutamate excitotoxicity via activation of P2Y receptors. *J Neurosci*, 2019; 39: 6038–48
42. Yu Linchen, Wang Ning, Zhang Yafan, et al: Neuroprotective effect of muscone on glutamate-induced apoptosis in PC12 cells via antioxidant and Ca<sup>2+</sup> antagonism. *Neurochem Int*, 2014; 70: 10–21
43. Garcia-Dorado D, Ruiz-Meana M, Inverte J et al: Calcium-mediated cell death during myocardial reperfusion. *Cardiovasc Res*, 2012; 94: 168–80
44. Chen XH, Chen DT, Huang XM et al: Dexmedetomidine protects against chemical hypoxia-induced neurotoxicity in differentiated PC12 cells via inhibition of NADPH oxidase 2-mediated oxidative stress. *Neurotox Res*, 2019; 35: 139–49
45. Yuan M, Meng XW, Ma J et al: Dexmedetomidine protects H9c2 cardiomyocytes against oxygen-glucose deprivation/reoxygenation-induced intracellular calcium overload and apoptosis through regulating FKBP12.6/RyR2 signaling. *Drug Des Devel Ther*, 2019; 13: 3137–49
46. Tseng EE, Brock MV, Lange MS et al: Glutamate excitotoxicity mediates neuronal apoptosis after hypothermic circulatory arrest. *Ann Thorac Surg*, 2010; 89: 440–45
47. Akpınar A, Uğuz AC, Nazıroğlu M: Agomelatine and duloxetine synergistically modulates apoptotic pathway by inhibiting oxidative stress triggered intracellular calcium entry in neuronal PC12 cells: Role of TRPM2 and voltage-gated calcium channels. *J Membr Biol*, 2014; 247: 451–59
48. Ganta KK, Mandal A, Chaubey B: Depolarization of mitochondrial membrane potential is the initial event in non-nucleoside reverse transcriptase inhibitor efavirenz induced cytotoxicity. *Cell Biol Toxicol*, 2016; 33: 69–82
49. Li Y, Zeng M, Chen W et al: Dexmedetomidine reduces isoflurane-induced neuroapoptosis partly by preserving PI3K/Akt pathway in the hippocampus of neonatal rats. *PLoS One*, 2014; 9: e93639
50. Cui J, Zhao H, Wang C et al: Dexmedetomidine attenuates oxidative stress induced lung alveolar epithelial cell apoptosis *in vitro*. *Oxid Med Cell Longev*, 2015; 2015: 358396
51. Liu XR, Li T, Cao L et al: Dexmedetomidine attenuates H<sub>2</sub>O<sub>2</sub>-induced neonatal rat cardiomyocytes apoptosis through mitochondria- and ER-mediated oxidative stress pathways. *Mol Med Rep*, 2018; 17: 7258–64
52. Wang K, Zhu Y: Dexmedetomidine protects against oxygen-glucose deprivation/reoxygenation injury-induced apoptosis via the p38 MAPK/ERK signalling pathway. *J Int Med Res*, 2018; 46: 675–86
53. Sanders RD, Sun P, Patel S et al: Dexmedetomidine provides cortical neuroprotection: Impact on anaesthetic-induced neuroapoptosis in the rat developing brain. *Acta Anaesthesiol Scand*, 2010; 54: 710–16
54. Jeon YT, Hwang JW, Lim YJ et al: Postischemic sevoflurane offers no additional neuroprotective benefit to preischemic dexmedetomidine. *J Neurosurg Anesthesiol*, 2013; 25: 184–90