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Zingiber officinale attenuates 6-hydroxydopamine induced oxidative stress and apoptosis through AKT, Nrf2, MAPK, NF-κB signaling pathway in PC12 cells

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Abstract

To explore the protective impact of the ginger root ethanol extract (GRE) against the oxidative stress induced by 6-hydroxydopamine (6-OHDA) and apoptotic and its mechanism. Parkinson's disease (PD) model was established using 6-OHDA in the cells of rat adrenal pheochromocytoma (PC12). The GRE pretreatment increased PC12 cell viability of injury induced by 6-OHDA. The GRE effectively suppressed 6-OHDA-induced death, apoptosis through decreased Bax and cleaved-caspase 3 expression, and up-regulated expression of B-cell lymphoma 2 (Bcl-2). GRE also inhibited 6-OHDA-inducel oxidative stress, decreased reactive oxygen species (ROS) and up-regulates the heme oxygenase (HO-1), antioxidant enzymes, catalase and, glutathione (GSH), superoxide dismutase (SOD), 8-oxoguanine glycosylase1 (OGG1) and NAD(P)H quinone oxidoreductase1 (NQO1). Besides, GRE up-regulating Protein kinase B (Akt), nuclear erythroid 2-related factor 2 (Nrf2), down-regulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) translocation, together with the mitogen-activated protein kinase (MAPK) phosphorylation. In conclusions, the GRE prevented apoptosis and oxidative stress in 6-OHDA-induced PC12 cells. The ginger ethanol extract could be treatment and prevention of PD.

Keywords: *Zingiber officinale*; Parkinson's disease; 6-hydroxydopamine; apoptosis; oxidative stress.

Practical Application: Ginger can be used for seasoning and traditional Chinese medicine, and can also be processed into health food to prevent aging and Parkinson's disease.

1 Introduction

Parkinson's disease (PD) is the second prevalent neurodegenerative motor disease in addition to Alzheimer's disease (AD). It is characterized via the degeneration or loss of the dopaminergic neurons in midbrain substantia nigra, leading to motor disorders, containing rigidity, tremor, postural instability and motor retardation (Blesa et al., 2012). PD related neurons apoptosis and degeneration is related to inflammation, the dysfunction of mitochondria, oxidative stress, oxidative DNA injury and the aggregation of α-synuclein. Furthermore, it is reported that oxidative stress markers increase and the activity of antioxidant enzyme decreases in patients with PD (Farooqui & Farooqui, 2011; Perier et al., 2012). ROS is fundamental for physiological cellular including cellular signaling and synaptic plasticity, but accumulate excessively in the brain leads to oxidative stress and neurons cells apoptotic death (Beckhauser et al., 2016). Glutathione peroxidase (GPx), catalase and SOD are crucial enzymes involving in the protection of neurons against ROS (Xiao et al., 1999). GSH has important anti-oxidation function (Dringen, 2000). PI3K/Akt signaling pathway can upregulating nuclear translocation of Nrf2 to the Nrf2/ARE pathway activation (Long et al., 2021). ARE/Nrf2 inducing antioxidant defense genes to scavenging ROS (Loboda et al., 2016). Akt, NF-κB, MAPKs pathway can regulating capoptosis of dopaminergic neurons (Long et al., 2021). Thus, using compounds with reduce oxidative stress and apoptosis effects fight against neurodegenerative disease.

6-OHDA is a typical neurotoxin, which can be employed as a PD model to induce the dopaminergic neurons death in *vitro* and in *vivo* (Blesa et al., 2012). PC12 cells have many performances similar to the dopamine neurons, and can be applied to investigate neuronal signaling pathways and secrete dopamine neurotransmitter in neurodegenerative diseases (Greene & Tischler, 1982; Seow et al., 2017). Studies have demonstrated 6-OHDA was induced in the PC12 cells as the PD experimental model in *vitro* (Kwon et al., 2010).

Ginger is the rhizomes of *Zingiber officinale*, is a common and extensively applied medicine and food for a long time (Mao et al., 2019). Chinese proverb says "eating turnip in winter and ginger in summer can keep doctors away". That is because ginger has prevention and it can treat various prevalent diseases, for instance, soothing the stomach, colds, dysmenorrhea, nausea, carsickness, and headaches (Mao et al., 2019). Recently, ginger and its compounds has been confirmed to bio-activities, contain antioxidant (Stoilova et al., 2007), anti-inflammatory (Ezzat et al., 2018), anticancer (Habib et al., 2008), neuroprotective (Mohd Sahardi & Makpol, 2019; Seow et al., 2017), antiobesityactivities

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(Kim et al., 2018). Previous studies found that 6-shogaol neuroprotective effects on the oxidative stress induced with 6-OHDA in the PC12 cells (Kabuto et al., 2005; Peng et al., 2015). However, the neuroprotective impact of ginger extract against the 6-OHDA neurotoxicity together with its latent mechanism are not clear. In this paper, we explored the influence of GRE on anti-apoptotic, antioxidant and its mechanisms on the PC12 cells induced with 6-OHDA.

2 Materials and methods

2.1 Reagents

6-OHDA, [6]-Gingerol (6-G) was provided by Sigma-Aldrich (St. Louis, MO, USA). Fetal bovine serum (FBS), horses serum (HS), Roswell Park Memorial Institute (RPMI) 1640 medium, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), Hanks' balanced salt solution (HBSS), 10000 U/mL Penicillin-Streptomycin were acquired from (GIBCO BRL, Grand Islang, NY, USA). The carboxy-H2DCFDA probe came from Invitrogen (Carlsbad, CA, USA). Quanti-Max™ form WST-8 cell viability assay kit, OxiTec™ catalase assay kit, mild protein extraction buffer, stripping buffer were supplied from (Biomax, Seoul, Korea). From Cayman Chemical (Ann Arbor, MI, USA), we can acquire the Glutathione (GSH) and Superoxide Dismutase (SOD) assay kit. Dimethyl sulfoxide (DMSO), antibodies for β-actin, NF-κB, P-Akt, Akt, P-ERK1/2, P-NF-κB, JNK, p38, P-p38, P-JNK, OGG1/2, HO-1, NQO1, Nrf2, Bcl-2 and Bax were offered by Santa Cruz Biotechnology (Santa Cruz, CA, USA). ERK, Anti-lamin B1 and secondary antibodies combined with horseradish peroxidase were provided by Cell Signaling Technology (Danvers, MA, USA). Protein assay kit was purchased from Bio-Rad (Hercules, CA, USA). Poly-L-lysin (PDL) solution was obtained from Sigma-Aldrich (St. Louis, MO, USA). RIPA buffer, chemilumi-nescence western blotting detection reagents, NE-PER nuclear and cytoplasmic extraction reagents were provided by Thermo Scientific (Rockford, IL, USA).

2.2 Plant material and extract preparation

Ginger was purchased from Bongdong traditional market (Wanju-gun, Jeonbuk, Korea). The ginger was washed and cut into pieces, dried in a dry oven at 40 °C for 48 h. Ginger was extracted (20 g) in 50% ethanol (400 mL) for 2 days. The sample after extracted was filtered by utilizing the filter paper (0.45 μm, Advantec, Japan), evaporating the filtrate and freeze-driving it into powder in the freeze dryer. Maintained under a temperature of -20 °C.

2.3 Cell culture and treatment

The PC12 cells were provided by the ATCC (Manassas, VA, USA), which were cultivated in a medium of RPMI 1640, and added with 1% P/S, heat-inactivated 10% HS and 5% FBS, subsequently incubated in a 37 °C incubator with humidified 5% CO_2. They were cultured in coated with poly-L-lysin solution cell culture dishes (SPL Life Sciences, gveonggi-do, korea). PC12 cells (2×10^5 cells/mL) were cultured 24 h and pretreated with GRE (100, 200 μg/mL) or 100 μM 6-G for an hour prior to the stimulation of PC12 with 6-OHDA (125 μM). After 6-OHDA stimulation, the cells were incubated for either 24 h or half an hour based on the research to be performed.

2.4 Cell viability

WTS-8 cell viability assay kit was applied to detect the cell viability. 2×10^5 cells/mL PC12 were cultivated in a a culture plate (96-well) for one day. The treatment of cells was conducted with various concentrations of GRE or with 6-OHDA alone for one day, next, each well was added with WST-8 (10 μL), incubated for four hours, and at 450 nm, the absorbance can be determined. The absorbance correlated with the number of live PC12. Used to calculate the treated and untreated controls relative ratio of cell viability.

With the aim of investigating the protective effect of the GRE, 2×10^5 cells/mL PC12 were cultivated in a culture plate (96-well) for 24 h, and the cells were pretreated via GRE with various concentrations for 60 minutes, and exposed to the 6-OHDA (125 μM) for one day, subsequently each well was added with WST-8 (10 μL) and incubated for four hours, at 450 nm, the absorbance was measured. This experiment is repeated three times.

2.5 Intracellular ROS detection

2×10^5 cells/mL of PC12 cells were cultured 24 h and pretreated with GRE (100, 200 μg/mL) or 100 μM 6-G for 60 minutes prior to the stimulation of PC12 with 6-OHDA (125 μM). After 1 h, the cells cultured medium replaced to HBSS supplemented with 1% HEPES, added 5 μM H2DCFDA incubated in darkness for half an hour under a temperature of 37 °C. The cells could be collected and cleaned using the solution of DPBS. Flow cytometer was applied to analyze the cell suspension.

2.6 GSH, SOD, and catalase activity measurement

2×10^5 cells/mL of PC12 cells were cultured 24 h and pretreated with GRE (100, 200 μg/mL) or 100 μM 6-G for 60 minutes, and the stimulation of PC12 was carried out with 6-OHDA (125 μM) for twenty-four hours. The extraction of whole-cell protein was conducted utilizing mild protein extraction buffer in accordance with the guidelines of manufacturer. Then the catalase, SOD, and GSH were determined based on the per assay kit manufacturer's instruction.

2.7 Protein extraction and western blot

The cell nuclear and cytoplasmic protein, the extraction of whole-cell protein was conducted using NE-PER nuclear and cytoplasmic extraction reagents, RIPA buffer extraction reagents, respectively. The protein and extraction used Bradford's reagent to measure the proteins concentration. Each sample 15 micrograms protein mixed with 5× SDS-PAGE loading buffer (Biosesang) for five minutes under a temperature of 95 °C. Running on a gel of SDS-PAGE (15% or 10%) and transferred to the membranes of polyvinylidene fluoride (PVDF). The above membranes subsequently were cleaned and cultured in Blocking Buffer (10 mL, Bio-Rad, Japan) for 5 minutes blocking to reduces non-specific antibody

binding to reduce background. The membranes were cultured with a variety of primary antibodies at under shaking overnight under a temperature of 4 °C. After cleaning, the membranes were cultured through the secondary antibody combined with corresponding HRP at RT under shaking for two hours. After 5 washes, expose the membranes to the WestGlow™ FEMTO Chemiluminescent substrate reagent (Biomax Co, Ltd, Seoul, Korea), which were then visualized on the UV detection imaging system (Alliance version 15.11; UVITEC, Cambridge, UK). The stripping buffer was utilized to strip the above membranes, and refilled them by other primary antibodies applying the above protocol. For each band, the density was detected using the software of ImageJ.

2.8 Statistical analysis

Student's t-test was applied for analyzing the data. Setting p of < 0.001, 0.01, and < 0.05 reflects significant outcomes statistically.

3 Results and discussion

3.1 Effects of GRE on the cell viability and apoptosis in 6-OHDA-induced PC12 cells

Firstly, the cytotoxic effect of GRE in the PC12 cells was determined. Shown in Figure 1A, the results indicated that GRE at concentrations ≤ 200 μg/mL did not display cytotoxicity, but at 400 μg/mL has slight cytotoxicity, cell viability 82%. Then the cell death induced through 6-OHDA was examined. According to Figure 1B, 6-OHDA can induce the toxicity of PC12 cells at concentrations of ≥100 μg/mL in concentration dependent manners. The cell viability after treated by the 6-OHDA (100, 125, 250 μM) were 80.1%, 64.0%, 35.2%, respectively. So, 6-OHDA (125 μM) for all the following researches was selected.

To explore whether GRE has a protective impact against the cell death induced via 6-OHDA. In accordance with Figure 1C, GRE pretreatment remarkably enhanced the cell viability of injury induced by 6-OHDA. The rate of cell viability for 6-OHDA only treated cells for one day was 65.2%. While pretreatment with 50 μg/mL, 100 μg/mL, 200 μg/mL together with 400 μg/mL of GRE increased cell viability to 68.6%, 70.4%, 87.7%, and 99.0%, respectively. Given up results, the GRE has effectively protect PC12 cells on the cell death induced via 6-OHDA with a concentration dependent mode. Also, RGE 100, 200 μg/mL and 6-OHDA were applied in the following studies.

Caspase-3 is a kind of protease, which is an apoptosis marker and the ultimate executor of the apoptotic DNA damage. The previous study have confirmed that 6-OHDA induced neuronal death in the PD is related to the caspase-3 activation (Wang et al., 2005). The family proteins of Bcl-2 are regarded as the principal regulators of the mitochondrial apoptosis pathway and has preventing apoptosis function (Wang et al., 2021). It is reported that the Bcl-2 prevents apoptosis with the modulation of antioxidant pathway. The excessive expression of Bcl-2 causes decreased oxidative stress leads to protects cells from apoptosis (Si et al., 2013). Bax is the pro-apoptotic gene in the family of Bcl-2. Reduce the ratio of Bax/Bcl-2 may promote neuronal apoptosis. Any agent that reduces the ratio of Bcl-2/Bax may facilitate apoptosis (Wei et al., 2005).

The cleaved-caspase 3 together with ratio of Bax/Bcl-2 were quantified to evaluate the GRE 6-OHDA-induced apoptosis through Western blot. In the Figure 1D-F, 6-OHDA-induced group showed that up-regulated expression of Bax protein and cleaved-caspase 3 and reduced Bcl-2, the Bax/Bcl-2 ratio levels up-regulated compared with non-stimulated cells. It suggested that the 6-OHDA results in the apoptosis of PC12 through the regulation of Bax, cleaved-caspase 3 and Bcl-2 expression levels. The GRE pretreatment remarkably downregulated the 6-OHDA induced ratio of Bax/Bcl-2 along with cleaved-caspase 3 with the dose-dependent mode. GRE can alteration in Bcl-2/Bax ratio leads to inhibits apoptosis.

3.2 Effects of GRE on ROS and SOD, GSH, catalase activity in 6-OHDA-induced PC12 cells

In contrast to other organs, the brain is more likely to affect by oxidative stress owing to the brain cells of human consume 20% oxygen despite the brain only accounts for 2% of body (Dringen, 2000). The intracellular ROS levels were ROS production caused by insufficient antioxidant defenses in neurodegenerative diseases. Excess ROS would damage proteins, mitochondrial, DNA and lipids, leading to neuronal injury and cell death (Chen et al., 2012). The scavenging ability of GRE on the creation of intracellular ROS induced via 6-OHDA in the PC12 cells was detected. Based on Figure 2A, the levels of ROS in the cells were evidently up-regulated in the PC12 induced by 6-OHD, whereas after GRE pretreatment, the levels of intracellular ROS were obviously reduced with the concentration-dependent mode. These results suggest that the GRE prevented the oxidative stress in the PC12 cells induced through 6-OHDA.

The activities of enzyme defense systems for example GSH, SOD and catalase are low, resulting in oxidative stress caused via the up-regulated ROS accumulation (Dringen, 2000). Cells are generally equipped with many antioxidant enzymes, for instance, GSH, SOD and catalase, as the detoxification systems for the prevention of damage resulted from ROS (Xiao et al., 1999). SOD catalyzes superoxide dismutation O_2- into H_2O_2 and O_2, playing as key enzymes of cellular antioxidant defense system, while catalase or GSH-Px subsequently decomposition H_2O_2 into O_2 and H_2O (Shim & Kim, 2013; Wang & Sun, 2017). Therefore, SOD, catalase and GSH as protective markers of oxidative stress. To determine whether pretreatment with GRE suppressed the oxidative stress in the PC12 cells induced by 6-OHDA, the antioxidant enzymes containing GSH, catalase and SOD were investigated. In accordance with Figure 2B, C, D, treatment by 6-OHDA evidently reduced cellular SOD, catalase and GSH activity comparison to the control, while the levels of GSH, catalase and SOD were remarkably up-regulated ($p < 0.01$) in pretreatment with GRE or 6-G. GRE markedly up-regulated the activity generation of GSH, catalase and SOD with the dose-dependent mode. The level of SOD, catalase and GSH activity to pretreatment with GRE (200 μg/mL) was higher than 6-G group. There is more and more evidence that the oxidative stress can cause the death of nerve cell in patients with Parkinson's disease. Recently, scientific work has begun to sought natural

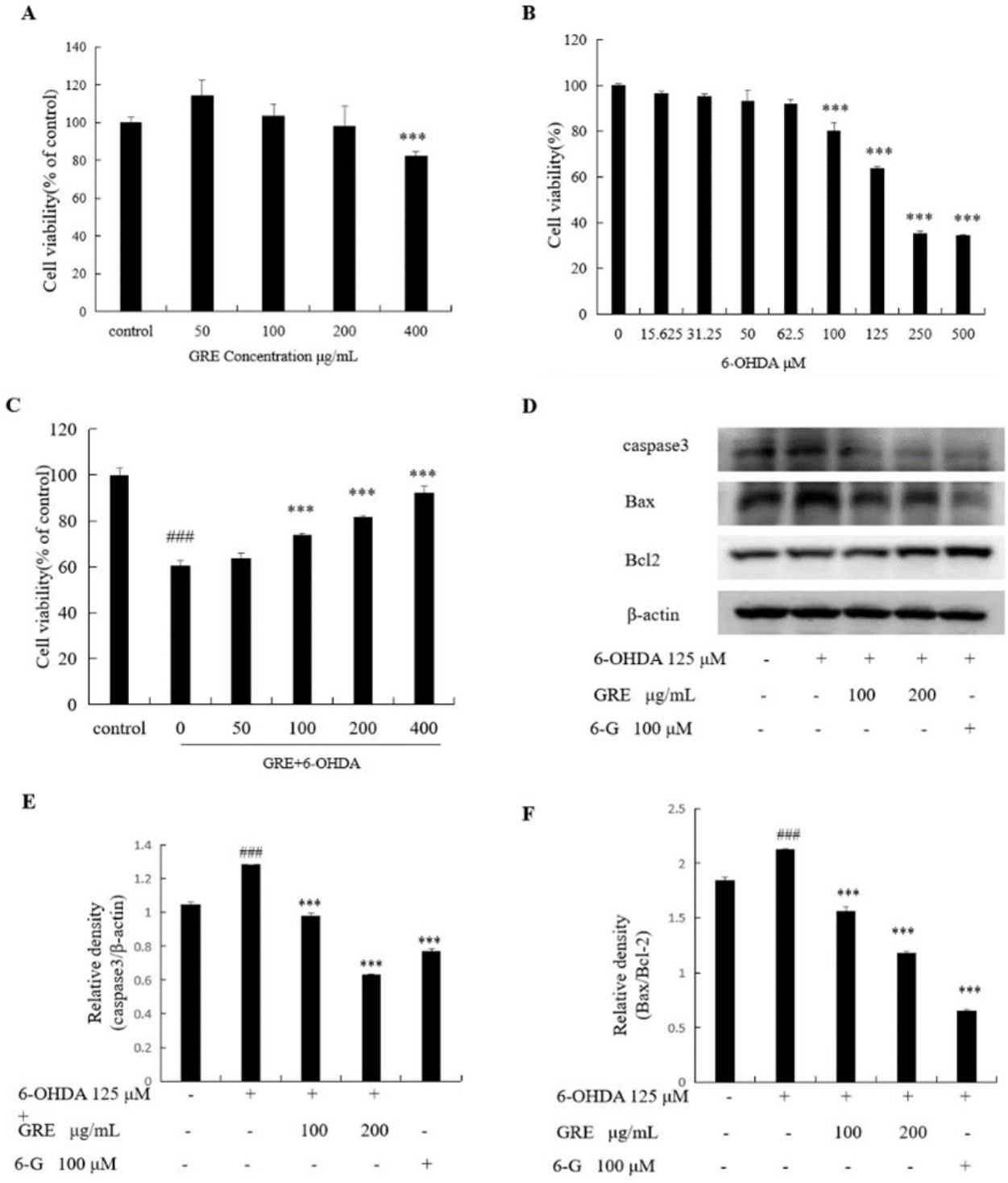

Figure 1. The effects of the GRE against cell apoptosis and viability in PC12 cells induced via 6-OHDA. 2×10^5 cells/mL of PC12 cells could be preprocessed through utilizing GRE with diverse concentrations for 60 minutes and then stimulated without or with the 6-OHDA for one day, then measured the cells viability using MTS assay and analyzed the Bcl-2, Bax together with cleaved-caspase 3 protein expression through the western blot assay. (A) Percentage of viable cells at diverse GRE concentrations; (B) 6-OHDA reduced cell viability; (C) GRE decreased 6-OHDA-induced cell cytotoxicity; (D) Bcl-2, Bax, cleaved-caspase 3 western blots; (E) protein expression relative to β-actin of cleaved-caspase 3; (F) the ratio of Bax/Bcl-2. Error bars represent the means ± SDs (n=3). ### $p < 0.001$ vs. control group, *** $p < 0.001$ vs. only 6-OHDA group.

medicines to antioxidant and anti-inflammatory properties, which may be effective in treating PD (Pohl & Kong Thoo Lin, 2018). So, the GRE can inhibit apoptosis and oxidative stress in the PC12 cells induced with 6-OHDA via catalase and SOD activity. They are also might berelated to the mechanisms of the neuroprotective effects of the GRE.

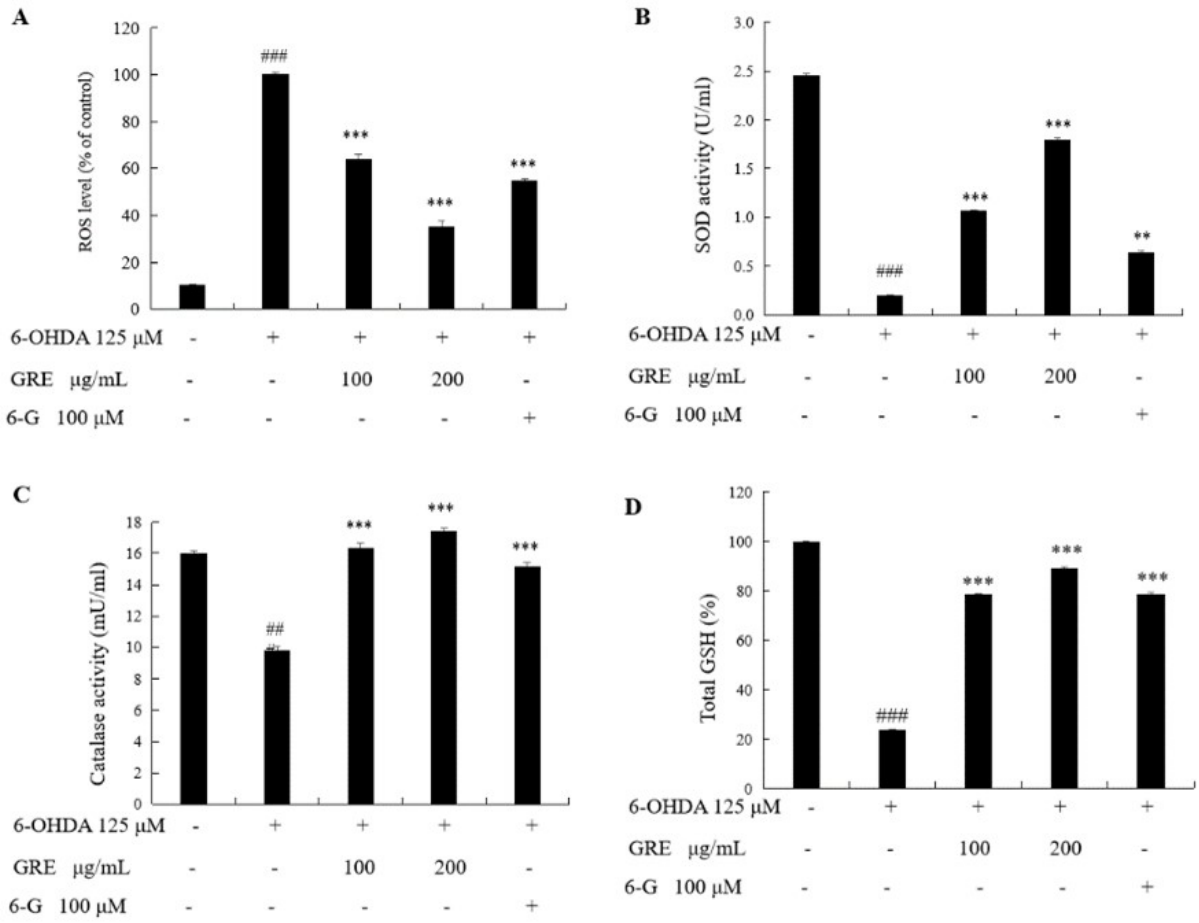

Figure 2. The Effects of the GRE suppressed the oxidative stress induced via 6-OHDA in the PC12 cells. Flow cytometry was employed to measure the level of ROS. SOD, catalase and GSH levels measured by kits. (A) the level of ROS; (B) the level of SOD; (C) the level of Catalase; (D) the level of GSH. Data were described with mean ± standard deviation (n = 3). $^{###}$ $p < 0.001$ vs. control group. *** $p < 0.001$ vs. only 6-OHDA group.

3.3 GRE induces Nrf2 nuclear translocation and HO-1, NQO1, and OGG1 expression in PC12 cells

Nrf2 possesses an essential effect in the oxidative stress neuroprotection with the regulation of antioxidant molecules and enzymes (Xu et al., 2021). Under general conditions, Nrf2 is isolated in cytoplasm. Oxidative stress can facilitate Nrf2 transport to nucleus and eventually causes its downstream targe antioxidant enzyme genes for example HO-1, NQO1 (Loboda et al., 2016).

Thus, to examined whether GRE enhance the nuclear translocation of Nrf2 and NQO1, HO-1 expression via the western blot assay. As reflected in the Figure 3A, B, protreatment with GRE or 6-G were significantly increase the nuclear Nrf2 expression. The upregulation expression of Nrf2 leads to increase in HO-1, NQO1 expression levels consistent with previous studies. The results suggesting that mechanism of neuroprotective effects of GRE related to Nrf2 nuclear translocation and modulation of antioxidant enzymes. So, GRE could positively affects the neuroprotective activity together with oxidative stress in the neurodegenerative diseases.

The PD, AD patients are related to the up-regulated oxidative stress, higher levels of 8-oxoguanine (8-oxoG) oxidative DNA damage (Mao et al., 2007; Nakabeppu et al., 2007). DNA repair defects cause the dysfunction of mitochondria. OGG1 is a significant DNA repair enzyme. Through the action of 8oxoG and OGG1, oxidative DNA injury can be minimized. OGG1 acts as a major pathway preventing the accumulation of 8-oxoG in the cellular DNA can suppress neurodegeneration (Mao et al., 2007).

The PC12 cells were exposed to 6-ODHA and led to the OGG1 degradation in Figure 3A, E. However, the cells that were pretreated with GRE before treatment with the 6-OHDA exhibited a remarkably up-regulated OGG1 level in comparison with the cells treated through using the 6-OHDA only. Nevertheless, pretreated by GRE revealed an evidently increased OGG1 level in comparison with 6-OHDA only treated. OGG1 could preventing 8-oxoG accumulation in cellular DNA, therefore, GRE also prevented DNA damage and oxidative stress in the neurodegenerative diseases.

3.4 Effects of GRE on MAPKs signaling pathway in 6-OHDA-induced in PC12 cells

MAPKs mediate extracellular stimuli into cellular responses and fundamental bio-processes, for instance the growth,

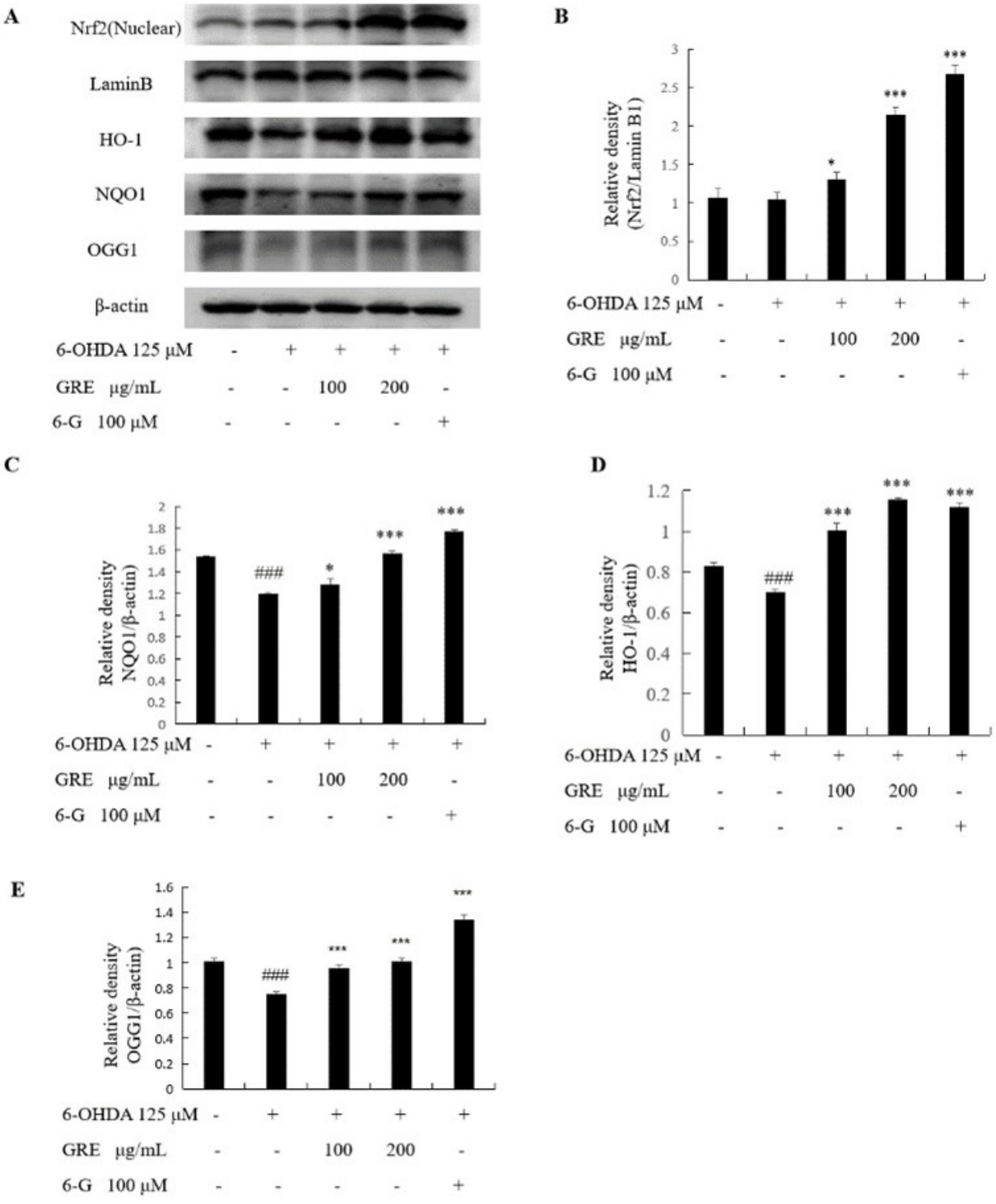

Figure 3. The effects of the GRE against HO-1, Nrf2, OGG1 and NQO1 protein expression induced with 6-OHDA in the PC12 Cells. PC12 cells were preprocessed using 100, 200 μg/mL of GRE and 100 μM of 6-G for 60 minutes, and then incubated by 125 μM 6-OHDA for one day. Whole-cell protein or nuclear proteins lysates were generated for western blot detection to test the protein levels of HO-1, Nrf2, OGG1 and NQO1. (A) The Nrf2 and HO-1, NQO1, OGG1 western blots, β-actin or Lamin B1 was employed as the internal control; (B) Nrf2 expression levels; (C) HO-1 expression levels; (D) NQO1 expression levels; (E) expression levels of OGG1. Error bars represent the means ± SDs. $^{###}p < 0.001$ vs. control group. $^{*}p < 0.05$, $^{***}p < 0.001$ vs. only 6-OHDA group.

proliferation, migration and differentiation, inflammation as well as apoptosis of the cell (Plotnikov et al., 2011). Studies reported that MAPK pathway is related to the apoptosis, cellular DNA injury and the oxidative stress (Rezatabar et al., 2019). Increasing evidences indicated that the stress kinase JNK to promote 6-OHDA-induced apoptosis (Mnich et al., 2010).

However, inhibition phosphorylation of p38 would associated with promote dopaminergic cell survival (Feng et al., 2019).

According to Figure 4A, B, D, 6-OHDA treatment increase the JNK, ERK phosphorylation compared with the control in PC12, while the pretreatment with GRE or 6-G was reduce. 6-OHDA treatment reduced the p38 phosphorylation, and the pretreatment with GRE or 6-G was increase p38 phosphorylation in Figure 4A, C. These results suggested that phosphorylation of JNK, ERK may promotes cell apoptosis while p38 phosphorylation promotes cell growth, GRE was neuroprotective in 6-OHDA via inhibition of JNK, ERK phosphorylation and activation of MAPK/p38 phosphorylation in PC12.

3.5 Effects of GRE on Akt/ NF-κB signaling pathway

NF-κB pathway activation is related to the oxidative stress and apoptosis in PD. NF-κB is normally located in cytoplasm, when the inflammatory response occurs or oxidative stress, NF-κB translocated to the nucleus (Hunot et al., 1997). Study have confirmed that the NF-κB signaling blockade is protected the PC12 cells death induced through 6-OHDA (Dong et al., 2015).

With the aim of further characterizing the mechanism via which GRE suppresses the oxidative stress together with apoptosis in the PC12 cells induced through 6-OHDA, NF-κB of cytoplasm and nucleus was investigated via the western blot detection. 6-OHDA highly markedly reduce NF-κB protein expression in cytoplasm and GRE or 6-G enhanced NF-κB protein expression (Figure 5A). However, protreatment with GRE or 6-G significantly suppressed the translocation of NF-κB induced with 6-OHDA from cytoplasm to the nucleus (Figure 5 B). The results demonstrate that GRE protection apoptosis and oxidative stress induced through 6-OHDA via blockade of the NF-κB signaling.

The signaling pathway of PI3K/Akt possesses an essential effect in oxidative stress and neuronal apoptosis. PI3K/Akt signaling pathway also related to pathogenesis of PD. The activated Akt can upregulating the Nrf2, Bcl-2 proteins expression to against cell apoptosis and oxidative stress induced with ROS (Long et al.,

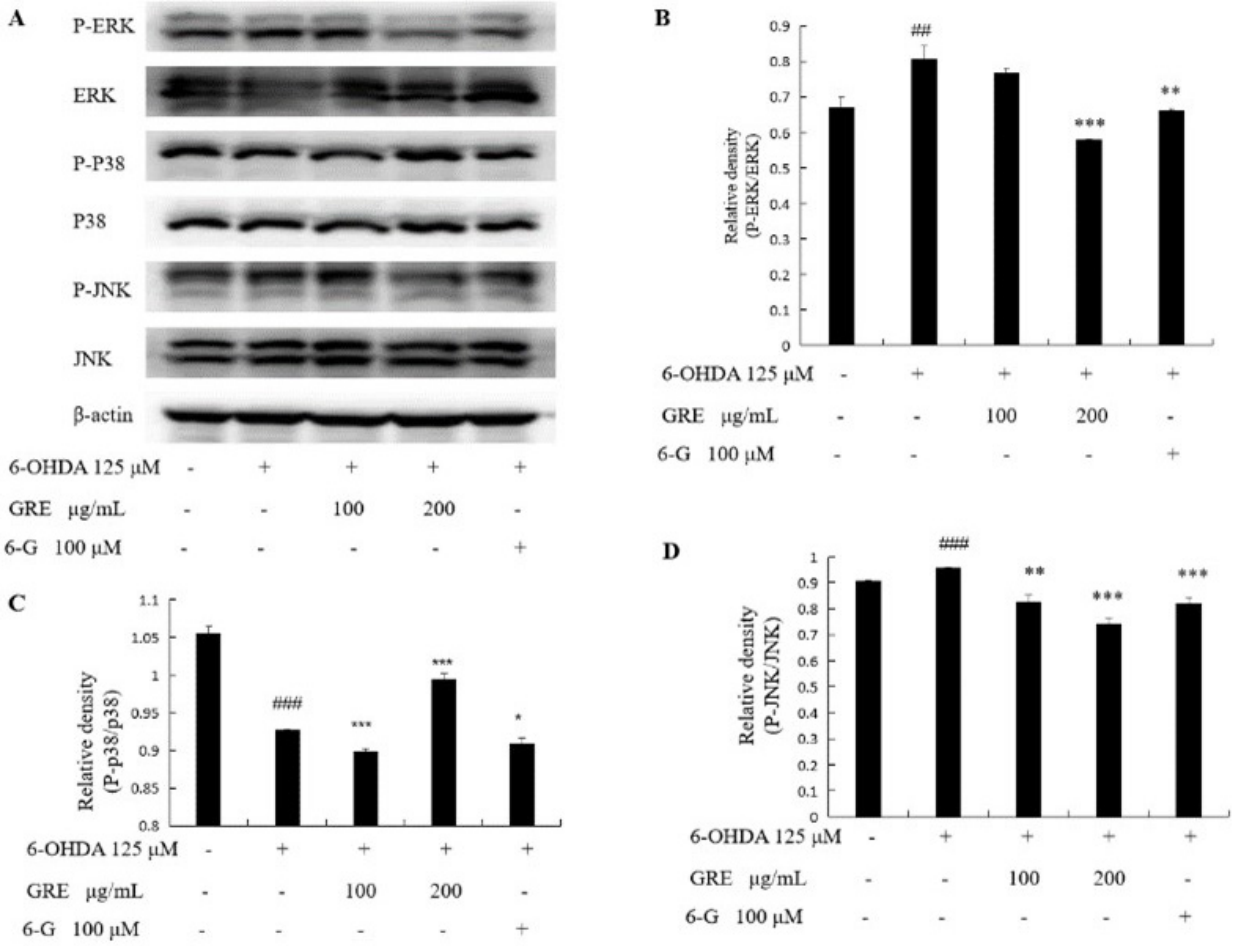

Figure 4. The effects of GRE against the MAPK signaling pathway in PC12 cells induced via 6-OHDA. PC12 cells were cultivated and then pre-treated by using 100, 200 μg/mL of GRE and 100 μM of 6-G for 60 minutes, and subsequently stimulated by 6-OHDA for half an hour. (A) The protein expression levels of phosphorylated or total forms of p38, ERK1/2, JNK cell signaling kinases were explored with the western blot detection, the software of ImageJ was applied for analyzing band densities, with respect to the β-actin; (B) P-ERK/ ERK expression levels; (C) P-p38/p38 expression levels; (D) p-JNK/JNK expression levels; Error bars represent the means ± SDs. $^{\#}$ $p < 0.05$; $^{\#\#}$ $p < 0.01$; $^{\#\#\#}$ $p < 0.001$ vs. control group. ** $p < 0.10$, *** $p < 0.001$ vs. only 6-OHDA group.

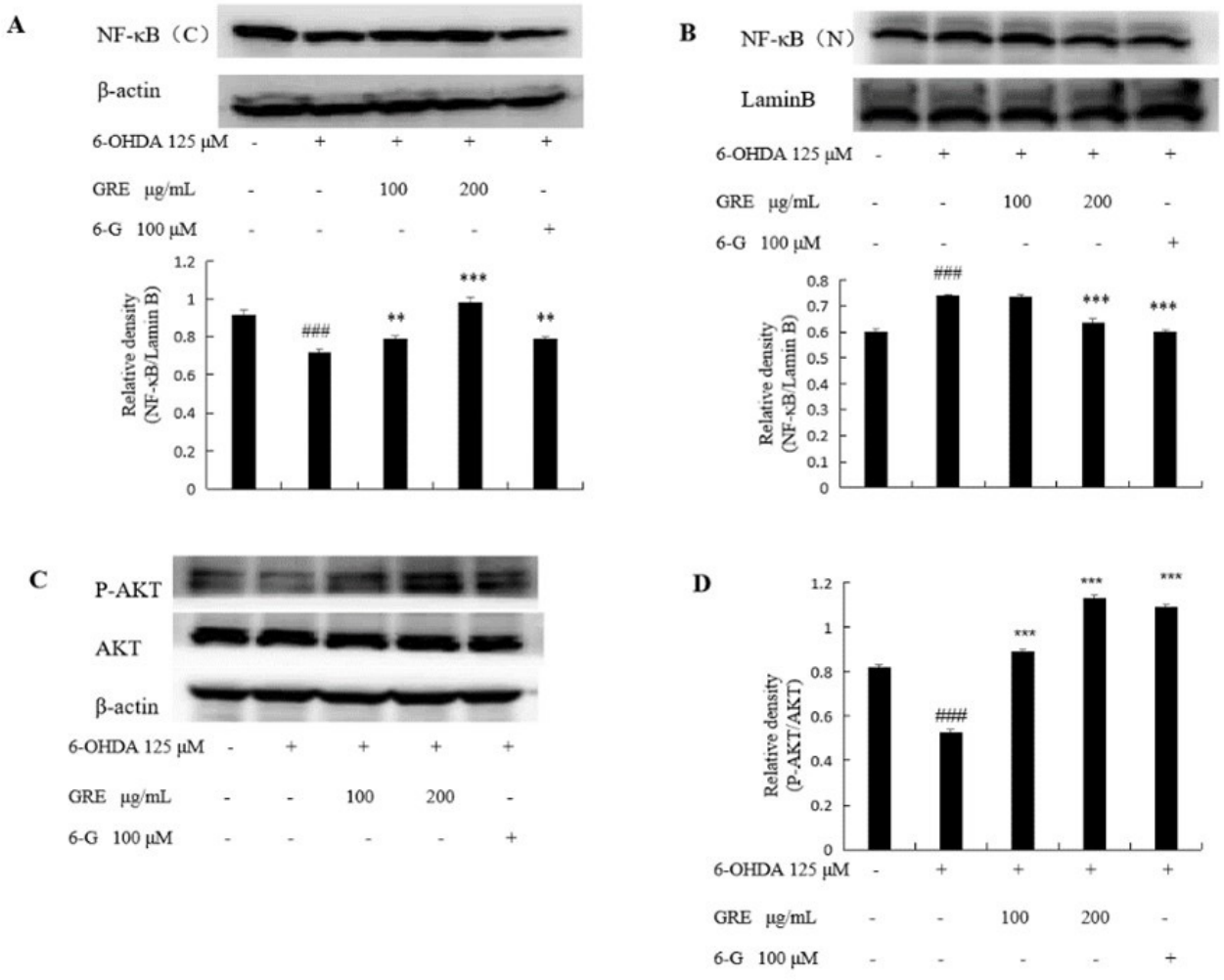

Figure 5. The effects of GRE against the Akt/NF-κB signaling pathway in PC12 cells induced via 6-OHDA. PC12 cells were cultivated and then pre-treated by using 100, 200 μg/mL of GRE and 100 μM of 6-G for 60 minutes, and subsequently stimulated by 6-OHDA for half an hour. Whole-cell nuclear or protein together with the cytoplasmic proteins lysates were created for the western blot detection. (A) NF-κB expression levels (nuclear); (B) NF-κB expression levels (cytoplasmic); (C) The Akt, and P-Akt western blots; (D) P-Akt/ Akt expression levels. Error bars represent the means ± SDs. $^{###}$ $p < 0.001$ vs. control group. ** $p < 0.10$, *** $p < 0.001$ vs. only 6-OHDA group.

2021). Thus, Akt and phosphorylated Akt protein expression levels was investigated with the western blot assay. As reflected in Figure 5C, D, 6-OHDA treatment decreased the P-Akt protein expression compared with the control in PC12. Furthermore, the pretreatment with GRE or 6-G significantly upregulation the levels of P-Akt/Akt ratio in the PC12 cells. These outcomes suggested that GRE may anti-oxidative and anti-apoptosis effect against the PC12 cells induced through 6-OHDA with the Akt signaling activation.

4 Conclusions

In this study the GRE showed comparable antioxidative and anti-apoptosis effects with 6-gingerol in PC12 cells induced via 6-OHDA. GRE have effectively protect cell death and oxidative damage induced by 6-OHDA via scavenging ROS and enhancement of the antioxidant enzymes for instance GSH, SOD and catalase. Also, GRE can activating Nrf2 signaling pathway, leads to increase of antioxidant enzyme, such as HO-1, NQO1. GRE ameliorates PC12-mediated neuronal insults through decreased cleaved-caspase 3, Bax/Bcl-2 and increase OGG1. GRE significantly attenuates the JNK, NF-κB translocation and ERK phosphorylation induced with the 6-OHDA, and enhanced phosphorylation of Akt, p38 and MAPK. The Akt, MAPKs together with NF-κB are located upstream of the Nrf2, caspase-3, Bax, Bcl-2, which regulate apoptosis and oxidative stress in PC12 cells induced via the 6-OHDA. The MAPKs also regulate the pathways of NF-κB and Nrf2. These outcomes demonstrate that GRE regulated the activation of Akt, MAPKs, NF-κB and following regulated the Nrf2, caspase-3, Bax, Bcl-2 protein expression. Therefore, GRE could positively affects antioxidant and neuroprotective activity without serious side effects, which might contribute to the slow the progression and prevention of PD.

Conflict of interest

The authors declare no conflict of interests.

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