ACTA ABP BIOCHIMICA POLONICA

Vol. 69, No 2/2022
447–452
https://doi.org/10.18388/abp.2020_5963

Regular paper

Glycitein exerts neuroprotective effects in Rotenone-triggered oxidative stress and apoptotic cell death in the cellular model of Parkinson's disease

Na Dong[1] and Zhilong Yang[2]✉

[1]Department of Neurology, Tianjin Huanhu Hospital, Tianjin, 300350, China; [2]Department of Neurology, The Third Affiliated Hospital of Soochow University, Changzhou, 213003, China

Parkinson's disease (PD) is one of the prevalent neurodegenerative diseases among aging populations. Despite recent advancements, still, no therapy exists for the prevention of PD. Given the remarkable pharmacological properties of flavonoids, this study was designed to investigate the protective effects of a plant-derived flavonoid, glycitein in a cellular model of PD. Rotenone, a prevalently used pesticide, has been employed to study the pathology of PD. The results of the present study showed that Glycitein significantly ($P<0.05$) prevented the rotenone-induced inhibition of cell viability as evident from the MTT assay. Additionally, it was found that the increased ROS levels triggered by rotenone in human SK-N-SH neuroblastoma cells were significantly ($P<0.05$) diminished upon glycitein treatment. Glycitein treatment also restored the mitochondrial membrane potential of the rotenone treated SK-N-SH cells. These effects of Glycitein were mainly due to its ability to trigger the increased activity of the ROS scavenging enzymes such as GSH, SOD and CAT. The ATP estimates showed that the ATP levels were significantly ($P<0.05$) reduced in the rotenone treated SK-N-SH cells. Nonetheless, glycitein treatment could restore the ATP levels of the SK-N-SH cells in a dose-dependent manner. Rotenone has also been shown to induce apoptosis in human cells and the results of the annexin V/PI staining showed that glycitein exhibits remarkable potential to prevent in the rotenone triggered apoptosis in SK-N-SH cells. This was also accompanied by alteration in the expression of Bax, Bcl-2 and caspase-3 expression. Taken together, the study indicates that glycitein exhibits neuroprotective effects by preventing rotenone induce oxidative stress and apoptotic cell death. These findings point towards the use of glycitein in the management of neurodegenerative diseases such as PD.

Keywords: glycitein; parkinson; apoptosis; oxidative stress; flavonoids

Received: 15 October, 2021; revised: 08 November, 2021; accepted: 30 December, 2021; available on-line: 12 June, 2022

✉e-mail: zhilongyang76@gmail.com

Abbreviations: MMP, mitochondrial membrane potential; MTT, MTT 3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide; ROS, reactive oxygen species

INTRODUCTION

Flavonoids constitute a vast and diverse group of phenolic compounds which are evolutionarily conserved and ubiquitously distributed across the plant kingdom(Ayaz *et al.*, 2019). Throughout evolution, these metabolites have developed a wide array of physiological roles in plants (Bombardi *et al.*, 2018). Human beings have used flavonoid-rich plant-derived extracts for the management of several ailments. The antioxidant, anti-inflammatory and anti-cancer properties are well established in the literature (Gutierrez-Zepeda *et al.*, 2005; Khan *et al.*, 2012). Studies have proved that the properties of flavonoids may be attributed to their ability to scavenge reactive oxygen species (ROS). The production of ROS has been held responsible for the development of several diseases including neurodegenerative diseases (Stephenson *et al.*, 2017). Neurodegeneration is the constant and slow loss of neuronal cells in particular regions of the brain. Studies have shown that neuronal loss is the main pathological characteristic of neurodegenerative diseases such as Parkinson's disease (PD) (Azad *et al.*, 2011). The aging of the brain, oxidative stress, mitochondrial dysfunction, and initiation of apoptosis are some of the main features of neurodegenerative disease (Reddy & Beal, 2008). Although tremendous progress has been made in the field of brain research, the molecular pathogenesis of neurodegenerative diseases is yet to be fully understood. The diagnosis of the disease at the advanced stage and proper therapeutic interventions are essential to prevent the progression of neurodegenerative diseases (Dauer & Przedborski, 2003). Since times immemorial, flavonoid enriched nutraceuticals have been used to improve cognitive function and to prevent the onset of neurodegenerative diseases in human (Lv *et al.*, 2019). Studies have also stressed the usage of flavonoid-rich natural products for the prevention of neurodegenerative diseases including PD (Zhang *et al.*, 2002; Hwang *et al.*, 2012). PD is generally characterized by motor and cognitive impairment and prevalently presents as a sporadic human ailment (Khan *et al.*, 2010). Data from human post-mortem tissues has revealed reactive oxygen species (ROS), and apoptotic activity to be critical in the pathogenesis of PD (Zhang *et al.*, 2002), suggesting that compounds interfering both with ROS and apoptosis might exert protective effects. Given the known antioxidant activity of flavonoids, the present study was designed to evaluate the protective effects of glycitein in cellular mode of PD.

MATERIALS AND METHODS

Cell Culture

The SK-N-SH neuroblastoma cells were grown in (DMEM) 12 (1: 1), supplemented with 2 mM glutamine, penicillin (100U/mL), streptomycin (100 U/mL), gentamicin (100 μg/mL), and 10% (vol/vol) heat-inactivated

fetal bovine serum (FBS) (Sigma Chemicals Co, St Louis, USA), and the medium was changed every two days. Cells were maintained at 37°C in a CO2 incubator in a saturated humidity atmosphere containing 95% air and 5% CO_2. Rotenone and Glycitein were dissolved in fresh DMSO (0.05%) prior to each experiment.

The rotenone treated SK-N-SH cells were used as a cellular model of PD. Briefly, the SK-N-SH cells were treated with different concentrations of rotenone (0, 0.5, 5, 50, 100 and 200 nM) for 24 h. Rotenone induces ROS production and therefore rotenone-treated cells, SK-N-SH cells have also been previously used as a cellular model of PD (Tamilselvam *et al.*, 2013).

Cell viability assay

The effects of rotenone or glycitein against SK-N-SH neuroblastoma cells were determined by the execution of MTT (3-(4,5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide) viability assay. Briefly, cells were plated onto a 96-well plate with a density of 2×10^5 cells each well and were pre-cultured for 24 h. Afterward, each well was supplemented with different rotenone or Glycitein concentration. Following treatment, the cells were treated with MTT solution and subjected to incubation for 4 h. MTT addition results in the formation of formazan crystals that are finally dissolved in DMSO (dimethyl sulfoxide). For optical density measurements, absorbance was recorded at 570 nm with an ELISA plate reader (Bio-Tek Instruments, Winooski, VT).

Annexin V/PI staining assay

To determine the effects of rotenone and glycitein on the apoptosis of SK-N-SH neuroblastoma cells, annexin V/PI staining was performed. Briefly, SK-N-SH cells were cultured in 6-well plates for 12 h with incubation at 37°C. Then, these cells were treated with different doses of rotenone or Glycitein for 24 h. To determine the percentage of apoptotic cells, the cells were stained with annexin V/FITC and subjected to analysis with a flow cytometer.

ROS determination

The formation of intracellular ROS in the SK-N-SH cells was determined by ROS assay kit with DCFH-DA fluorescence probe. After rotenone or glycitein treatment, the SK-N-SH cells were administrated for 25 min with 10 μL of DCFH-DA. The non-fluorescent DCFH was converted to fluorescent DCF by intracellular ROS which was measured by an inverted fluorescence microscope. Subsequently, the cells were collected by centrifugation and the intensity of DCF fluorescence was estimated by fluorescence spectrophotometer at 488 nm to determine the ROS percentage.

MMP determination

Alteration in the MMP of the rotenone or glycitein treatment was examined by fluorescence using JC-1. Following rotenone or Glycitein treatment, the SK-N-SH cells were subjected to PBS washing and treated for 30 min with 5 μM JC-1.

Finally, inverted fluorescence microscopy was used to obtain the fluorescent images (Leica, Germany) at 514 nm.

Determination GSH, SOD and CAT

For the determination of the CAT, SOD and GSH, the cells were subjected to treatment with rotenone, glycitein or a combination of both. For enzyme activity, the cells were subjected to homogenization with extraction buffer followed by centrifugation at $18000\times g$ for 25 min. Thereafter, the supernatant was harvested and used for the determination of CAT activity. The reaction mixture consisted of 5 mM H_2O_2 in 50 mM PBS buffer (pH 7.0). The reaction was initiated by the addition of 100 μL of protein extract to 900 μL of reaction solution. The decrease in OD_{240} at room temperature was measured by spectroscopy. One unit of CAT was defined as the amount of enzyme that could decompose 1 μmol of H_2O_2 in 1 min at 25°C (extinction coefficient is 0.039 $M^{-1}cm^{-1}$at 240 nm).

For superoxide dismutase (SOD) activity each 3 mL of reaction mixture had 50 mM sodium phosphate (pH 7.8), 13 mM methionine, 75 mM nitroblue tetrazolium salt (NBT), and 0.1 mM EDTA. Small aliquots of this mixture were separately put into small glass tubes and addition of 100 μL of protein extract plus mM of riboflavin was followed. After thorough mixing the tubes were illuminated. In control tube the sample was replaced by 100 μL buffer. Change in absorbance by the formation of the formazan was measured at OD_{560}. The increase in the absorbance without the enzyme extract was taken as 100% and the enzyme activity was calculated by determining the percentage inhibition per minute. 50% inhibition was taken as equivalent to 1 unit of SOD enzyme.

For GSH activity, an aliquot of 50 μL of protein extract was added to 740 μL of sodium phosphate buffer (0.1 M; pH 7.4), 50 μL of 1 mM of sodium azide, 25 μL of glutathione reductase (1 unit/mL), 5 μL of 0.25 mM of H_2O_2, 25 μL of 1 mM GSH, 50 μL of 1 mM of EDTA and 50 μL of 0.2 mM of NADPH. Oxidation of NADPH (substrate) was determined at 340 nm. Activity of GSH was calculated as amount of NADPH oxidized per min per mg protein.

Estimation of ATP Levels

The treated SK-N-SH were collected by centrifugation at high speed. The levels of the intracellular ATP were then determined with the help of ATP Bioluminescence Kit HS II (Roche) as per the guidelines of the manufacturer.

Western blotting analysis

After treatment with rotenone or Glycitein doses, the SK-N-SH cells were lysed using a lysis buffer. Protein content within each lysate was quantified with a BCA assay. Afterward, 40 μg cell lysates were resolved through SDS-PAGE and then electrophoretically transferred to nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, United States). Thereafter, membranes were blotted using primary antibodies (Santa Cruz, CA, USA) with 1:1000 dilution. Then membranes were subjected to secondary antibodies treatment at 4°C overnight. Finally, an enhanced chemiluminescence reagent (ECL) (Amersham, Piscataway, NJ, United States) was utilized to spot the protein (Bax, Bcl-2, caspase-3 and actin) signals.

Statistical analysis

Experiments were done with three biological replicates and the values are shown as mean ± standard deviation (S.D.). Student's *t*-test (for comparison between two samples) and one way analysis of variance followed by Tukey's test (for comparison between more than two samples) were used for statistical analysis using Graph-

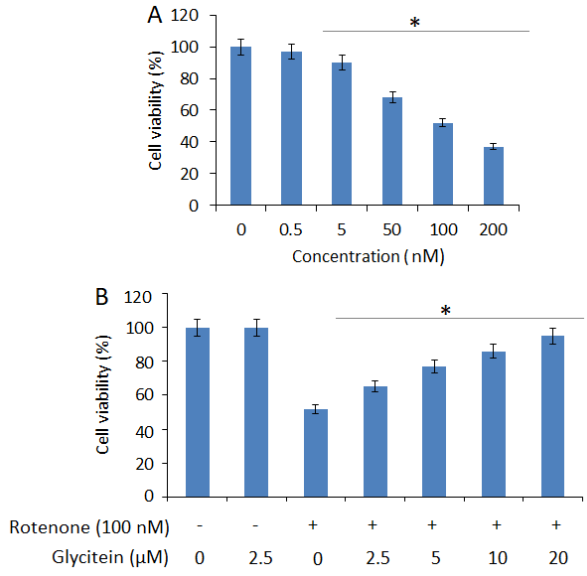


Figure 1. Glycitein prevents the rotenone-induced cell viability. (**A**) Effects of rotenone on the viability of SK-N-SH neuroblastoma cells (**B**) Effects of Glycitein on the rotenone-induced inhibition of cell viability. The experiments were performed in triplicate and expressed as mean ± S.D. (*P<0.05).

Pad Prism software (version 7; GraphPad Software, Inc., La Jolla, CA, USA. P<0.05 was taken as the measure of the statistically significant difference.

RESULTS

Glycitein prevents rotenone-induced decrease in cell viability

The results of the MTT cell viability assay showed that rotenone caused a significant (P<0.05) depletion of the cell viability of the human SK-N-SH neuroblastoma cells. These effects of rotenone were found to be dose-dependent. The IC_{50} of rotenone against human SK-N-SH cells was found to be 100 nM (Fig. 1A). Next, the SK-N-SH cells were treated with 0 or 100 nM rotenone plus different doses of glycitein (0, 2.5, 5, 10 and 20 μM). The results showed that glycitein caused a significant (P<0.05) restoration of the rotenone-induced depletion of SK-N-SH neuroblastoma cell viability (Fig. 1B).

Glycitein inhibits the rotenone-induced ROS formation and MMP loss

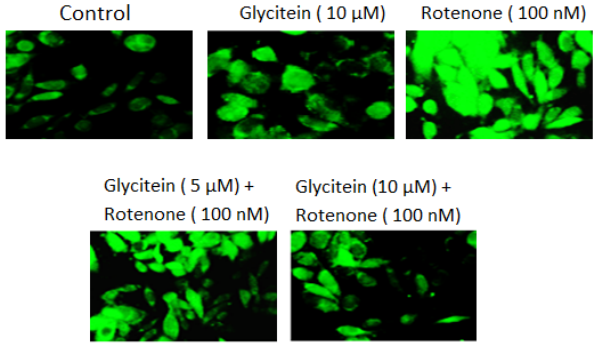
Next, the ROS levels of the rotenone treated (100 nM) and untreated cells were determined by the DCFH-DA fluorescence probe. It was found that rotenone caused a significant (P<0.05) increase of the ROS levels in SK-N-SH cells. The ROS levels increased to around 215% relative to the untreated control. Nonetheless, the ROS levels decreased significantly (P<0.05) and dose-dependently upon glycitein treatment as revealed by the intensity of the fluorescence (Fig. 2). The fluorescence probe JC-1was used to examine the MMP levels of the untreated and rotenone treated SK-N-SH cells. The results showed that rotenone caused a significant (P<0.05) decline in the MMP levels of the SK-N-SH cells relative to the untreated control. The MMP levels in the rotenone treated SK-N-SH cells decreased to around 20%. Nonetheless, glycitein treatment restored the mitochondrial function by increasing the MMP levels in rotenone-treated cells. The MMP levels were 55% and 67% at 5 and 10 μM doses of Glycitein (Fig. 3).

Glycitein increased the activity of the antioxidant enzymes

The effects of glycitein on the rotenone-induced suppression were assessed by standard biochemical assay. The results showed significant (P<0.05) depletion of the GSH, SOD and CAT proteins upon rotenone treatment of the SK-N-SH cells relative to untreated cells. However, glycitein treatment of human SK-N-SH cells caused a significant (P<0.05) and dose-dependent increase of the GSH, SOD and CAT (Fig. 4A–C).

Glycitein prevents rotenone induces ATP depletion

The ATP levels of the rotenone administrated cells were estimated by standard assay, and it was found that treatment of the SK-N-SH cells with rotenone caused a significant decrease of the ATP levels. The ATP levels of the SK-N-SH cells decrease to less than 40% of the untreated control. However, administration of glycitein to rotenone treated SK-N-SH cells could significantly (P<0.05) and dose dependently restore the ATP levels (Fig. 5).



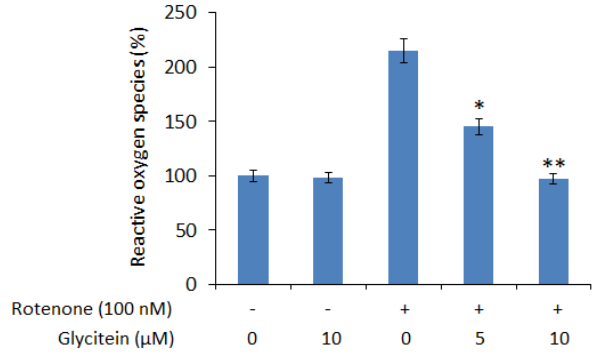


Figure 2. Glycitein inhibits the rotenone-induced ROS levels in neuroblastoma cells. The experiments were performed in triplicate and expressed as mean ± S.D. (*P<0.05 and **P<0.01).

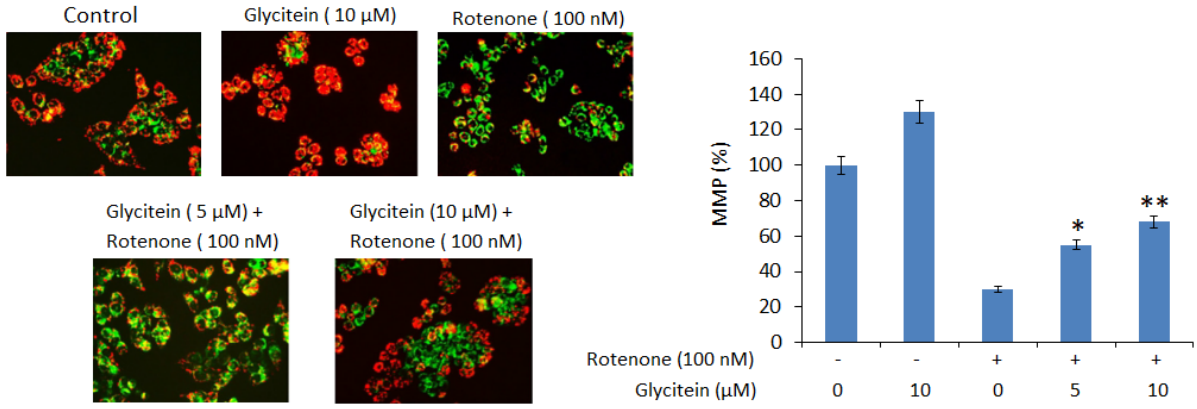


Figure 3. Glycitein restores the rotenone decrease in MMP levels in neuroblastoma cells.
The experiments were performed in triplicate and expressed as mean ±S.D. (*P<0.05 and **P<0.01).

Glycitein prevents rotenone-induced apoptosis

Annexin V/PI staining was performed to assess the effects of glycitein on the rotenone-induced apoptosis. The results showed that rotenone caused apoptosis of the SK-N-SH cells. The apoptotic SK-N-SH cell percentage was 21% relative at 100 nM dosage of rotenone. Glycitein at 5 μM treatment reduced the rotenone-induced apoptosis to 13%. At 10 μM dosage of the glycitein, the apoptosis decreased to 5.7% (Fig. 6A). The western blot analysis showed that glycitein treatment caused a decrease in a rotenone triggered upregulation of Bax and caspase-3 expression (Fig. 6B). This was concomitant with an increase in Bcl-2 expression indicative of the anti-apoptotic effects of glycitein on the SK-N-SH cells.

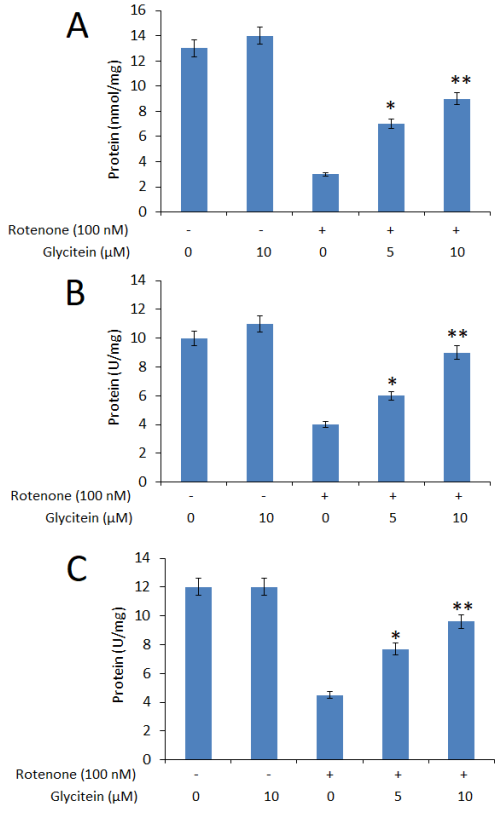


Figure 4. Effect of Glycitein on rotenone-induced decrease in (A) GSH (B) SOD and (C) CAT.
The experiments were performed in triplicate and expressed as mean ± S.D. (*P<0.05 and **P<0.01).

DISCUSSION

Oxidative stress, loss of mitochondrial membrane potential and initiation of apoptotic cell death are some important triggers in the development of PD (Moon *et al.*, 2005). Rotenone also triggers these events in the neuroblastoma cells and rotenone treated neuroblastoma cells were thus used to act as the cellular model of PD as has been done previously (Tamilselvam *et al.*, 2013). The present findings show that rotenone decreases the viability of the SK-N-SH cells which is consistent with previous studies (Lee *et al.*, 2010). Glycitein is a plant-derived flavonoid with tremendous pharmacological potential. It has been shown to exert protective effects against human glioma (Zang *et al.*, 2019). Zhang *et al.*, showed that glycitein induces apoptosis in human gastric cancer cells (Kang *et al.*, 2007). In another study, Kang and others (Kang *et al.*, 2007) showed that glycitein exerts protective effects on the hydrogen peroxide induced cell damage by scavenging reactive oxygen species. In yet another study, glycitein has been shown to inhibit the proliferation of breast cancer cells (Zhang *et al.*, 2015). Nonetheless, in the present study, we for the first time show that a natural flavonoid glycitein attenuates the toxic effects of rotenone. Rotenone has been shown to cause the production of ROS in human cells (Wang *et al.*, 2005; Radad *et al.*, 2006) and this was further validated by our fin-

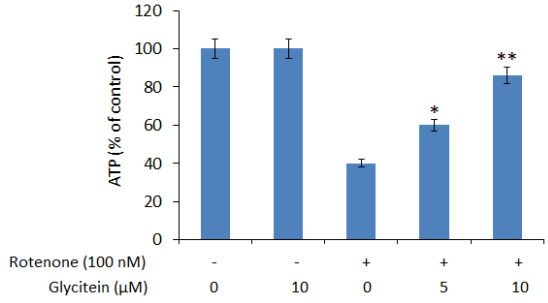


Figure 5. Effect of Glycitein on the rotenone-induced decrease in ATP levels of SK-N-SH neuroblastoma cells.
The experiments were performed in triplicate and expressed as mean ± S.D. (*P<0.05 and **P<0.01).

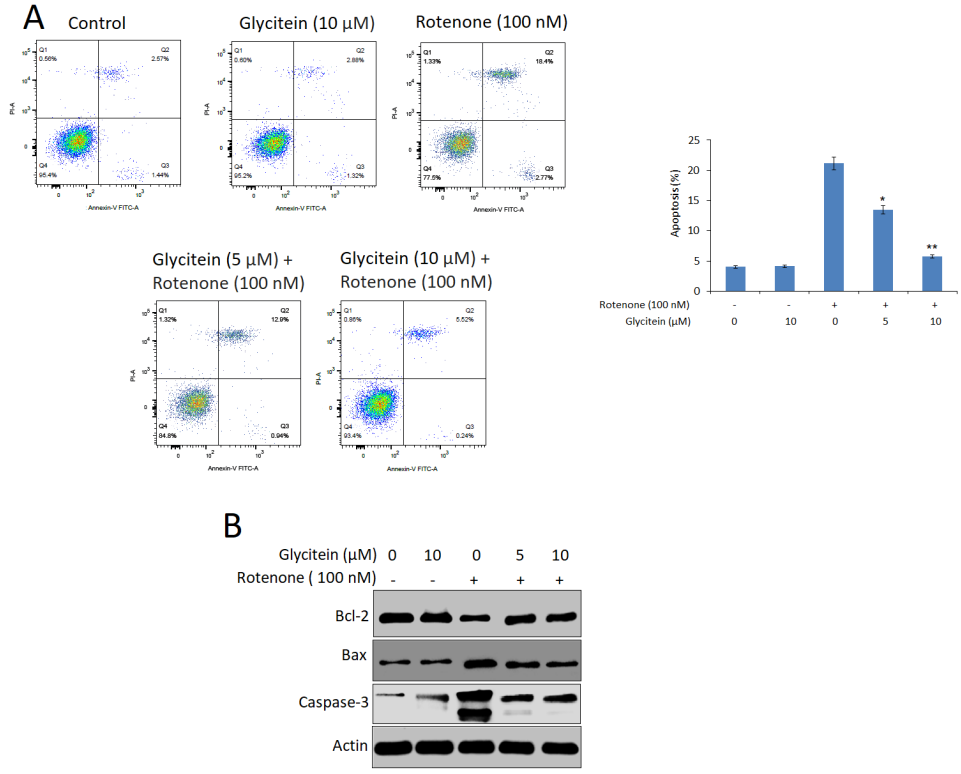


Figure 6. Glycitein prevents rotenone-induced apoptosis
(**A**) Effect of Glycitein on the rotenone-induced apoptosis in SK-N-SH neuroblastoma cells as depicted by annexin V/PI staining (**B**) Effect of Glycitein on the rotenone-induced alterations of Bax, Bcl-2 and Caspase-3. The experiments were performed in triplicate and expressed as mean ± S.D. (**P*<0.05 and ***P*<0.01).

dings wherein we show that ROS levels significantly increase upon rotenone treatment of neuroblastoma cells. Rotenone triggers the accumulation of the electrons by blocking the respiratory complex 1. These electrons are transferred to the oxygen molecules leading to the formation of O_2^- (Przedborski *et al.*, 2005). The oxidative stress is mainly manifested in the mitochondrial membrane, leading to the disruption of mitochondrial membrane potential. Herein, we found that glycitein could efficiently restore the mitochondrial membrane potential of the rotenone treated neuroblastoma cells. The decrease in the ROS and increase in the MMP levels in the neuroblastoma cells could also be attributed to the capacity of the glycitein to increase the activities of GSH, CAT and SOD. These enzymes efficiently scavenge ROS (Przedborski *et al.*, 2005), leading to a decrease in oxidative stress and consequently restoration of the mitochondrial membrane potential. All these findings are in agreement with previous studies wherein flavonoids such as hesperidin have attenuated the rotenone that triggered oxidative stress (Tamilselvam *et al.*, 2013). Additionally, a previously carried out study has shown that glycitein could attenuate amyloid-induced toxicity and oxidative stress (Khan *et al.*, 2012). ATP is considered essential for the induction of apoptosis (Eguchi *et al.*, 1999). However, rotenone caused depletion of ATP levels so that the induction of apoptosis by rotenone in the neuroblastoma cells could be due to some other mechanism such as oxidative stress. Glycitein on the other hand prevented the rotenone-induced apoptosis via reduction of oxidative stress and restoration of MMP. The reduction of MMP causes the discharge of cytochrome *c* which in turn leads to the activation of the caspase-3 favouring apoptotic cell death. Additionally, apoptosis is also accompanied by the enhancement of Bax and a decrease of the Bcl-2 expression levels (Jiang & Wang 2004). Herein, we found that glycitein inhibited apoptosis by reducing the expression of Bax, increasing the expression of Bcl-2 and by inhibiting the activation of caspase-3.

CONCLUSION

Taken together, the findings indicate that glycitein attenuates the rotenone-induced neuronal damage by scavenging ROS and thereby preventing oxidative stress and mitochondrial damage. Moreover, glycitein was found to prevent the induction of the apoptotic cell death in rotenone treated cells. These results point towards the applicability of glycitein in the prevention of PD via scavenging of ROS. Although the present study suggests the therapeutic potential of glycitein in the management of the PD, the results are yet preliminary and therefore *in vivo* studies are urgently required to further validate the neuroprotective effects of glycitein.

Competing interests

The authors declare no competing interests.

REFERENCES

Ayaz M, Sadiq A, Junaid M, Ullah F, Ovais M, Ullah I, Ahmed J, Shahid M (2019) Flavonoids as prospective neuroprotectants and their therapeutic propensity in aging associated neurological disorders. *Front Aging Neurosci* **11**: 155. https://doi.org/10.3389/fnagi.2019.00155

Azad N, Rasoolijazi H, Joghataie MT, Soleimani S (2011) Neuroprotective effects of carnosic Acid in an experimental model of Alzheimer's disease in rats. *Cell J* **13**: 39–44

Bombardi Duarte AC, Santana MG, di Camilo Orfali G, de Oliveira CTP, Priolli DG (2018) Literature evidence and ARRIVE assessment on neuroprotective effects of flavonols in neurodegenerative diseases' models. CNS *Neurol Disord Drug Targets* **17**: 34–42. https://doi.org/10.2174/1871527317666171221110139

Dauer W, Przedborski S (2003) Parkinson's disease: mechanisms and models. *Neuron* **39**: 889–909. doi: 10.1016/s0896-6273(03)00568-3.

Eguchi Y, Srinivasan A, Tomaselli KJ, Shimizu S, Tsujimoto Y (2004) ATP-dependent steps in apoptotic signal transduction. *Cancer Res* **59**: 2174–2181

Jiang X, Wang X. Cytochrome *c*-mediated apoptosis. *Annu Rev Biochem* **73**: 87–106. https://doi.org/10.1146/annurev.biochem.73.011303.073706

Gutierrez-Zepeda A, Santell R, Wu Z, Brown M, Wu Y, Khan I, Link CD, Zhao B, Luo Y (2005) Soy isoflavone glycitein protects against beta amyloid-induced toxicity and oxidative stress in transgenic Caenorhabditis elegans. *BMC Neurosci* **6**: 54. https://doi.org/10.1186/1471-2202-6-54

Hwang SL, Shih PH, Yen GC (2012) Neuroprotective effects of citrus flavonoids. *J Agric Food Chem* **60**: 877–885. https://doi.org/10.1021/jf204452y

Kang KA, Zhang R, Piao MJ, Lee KH, Kim BJ, Kim SY, Kim HS, Kim DH, You HJ, Hyun JW (2007) Inhibitory effects of glycitein on hydrogen peroxide induced cell damage by scavenging reactive oxygen species and inhibiting c-Jun N-terminal kinase. *Free Radic Res* **41**: 720–729. https://doi.org/10.1080/10715760701241618

Khan MM, Ahmad A, Ishrat T, Khan MB, Hoda MN, Khuwaja G, Raza SS, Khan A, Javed H, Vaibhav K, Islam F (2010) Resveratrol attenuates 6-hydroxydopamine-induced oxidative damage and dopamine depletion in rat model of Parkinson's disease. *Brain Res* **1328**: 139–151. https://doi.org/10.1016/j.brainres.2010.02.031

Khan MM, Raza SS, Javed H, Ahmad A, Khan A, Islam F, Safhi MM, Islam F (2012) Rutin protects dopaminergic neurons from oxidative stress in an animal model of Parkinson's disease. *Neurotox Res* **22**: 1–15. https://doi.org/10.1007/s12640-011-9295-2

Lee EJ, Kim SY, Hyun JW, Min SW, Kim DH, Kim HS (2010) Glycitein inhibits glioma cell invasion through down-regulation of MMP-3 and MMP-9 gene expression. *Chem Biol Interact* **185**: 18–24. https://doi.org/10.1016/j.cbi.2010.02.037

Lv C, Hong T, Yang Z, Zhang Y, Wang L, Dong M, Zhao J, Mu J, Meng Y (2012) Effect of quercetin in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine-induced mouse model of Parkinson's disease. *Evid Based Complement Alternat Med* 928643. https://doi.org/10.1155/2012/928643

Moon Y, Lee KH, Park JH, Geum D, Kim K (2005) Mitochondrial membrane depolarization and the selective death of dopaminergic neurons by rotenone: protective effect of coenzyme Q10. *J Neurochem* **93**: 1199–1208. https://doi.org/10.1111/j.1471-4159.2005.03112.x

Przedborski S, Ischiropoulos H (2005) Reactive oxygen and nitrogen species: weapons of neuronal destruction in models of Parkinson's disease. *Antioxid Redox Signal* **7**: 685–693. https://doi.org/10.1089/ars.2005.7.685

Radad K, Rausch WD, Gille G (2006) Rotenone induces cell death in primary dopaminergic culture by increasing ROS production and inhibiting mitochondrial respiration. *Neurochem Int* **49**: 379–386. https://doi.org/10.1016/j.neuint.2006.02.003

Reddy PH, Beal MF (2008) Amyloid beta, mitochondrial dysfunction and synaptic damage: implications for cognitive decline in aging and Alzheimer's disease. *Trends Mol Med* **14**: 45–53. https://doi.org/10.1016/j.molmed.2007.12.002

Stephenson J, Nutma E, van der Valk P, Amor S (2018) Inflammation in CNS neurodegenerative diseases. *Immunology* **154**: 204–219. https://doi.org/10.1111/imm.12922

Tamilselvam K, Braidy N, Manivasagam T, Essa MM, Prasad NR, Karthikeyan S, Thenmozhi AJ, Selvaraju S, Guillemin GJ (2013) Neuroprotective effects of hesperidin, a plant flavanone, on rotenone-induced oxidative stress and apoptosis in a cellular model for Parkinson's disease. *Oxid Med Cell Longev* **2013**: 102741. https://doi.org/10.1155/2013/102741

Wang G, Qi C, Fan GH, Zhou HY, Chen SD (2005) PACAP protects neuronal differentiated PC12 cells against the neurotoxicity induced by a mitochondrial complex I inhibitor, rotenone. *FEBS Lett* **579**: 4005–4011. https://doi.org/10.1016/j.febslet.2005.06.013

Zang YQ, Feng YY, Luo YH, Zhai YQ, Ju XY, Feng YC, Wang JR, Yu CQ, Jin CH (2019) Glycitein induces reactive oxygen species-dependent apoptosis and G0/G1 cell cycle arrest through the MAPK/STAT3/NF-κB pathway in human gastric cancer cells. *Drug Dev Res* **80**: 573–584. https://doi.org/10.1002/ddr.21534

Zhang B, Su JP, Bai Y, Li J, Liu YH (2000) Inhibitory effects of O-methylated isoflavone glycitein on human breast cancer SKBR-3 cells. *Int J Clin Exp Pathol* **8**: 7809–7817

Zhang Y, Dawson VL, Dawson TM (2000) Oxidative stress and genetics in the pathogenesis of Parkinson's disease. *Neurobiol Dis* **7**: 240–250. https://doi.org/10.1006/nbdi.2000.0319