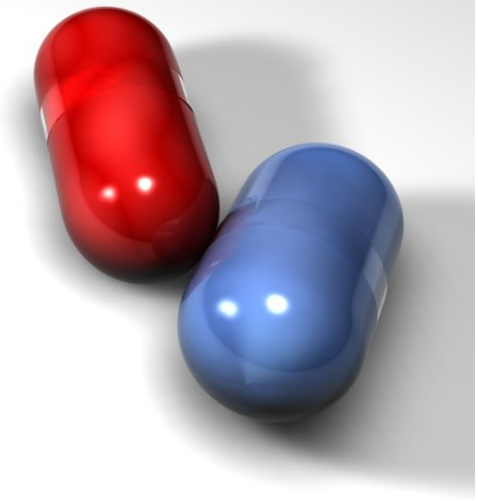
Bangladesh Pharmacological Society



Bangladesh Journal of Pharmacology

Volume: 21; Number 1; Year 2026

Cite this article as: Liu Q, Bao J, Ding RB. Neuroprotective effect of juglone against 6-OHDA-induced neurotoxicity in PC12 cells. Bangladesh J Pharmacol. 2026; 21: 35-38.

A Journal of the Bangladesh Pharmacological Society (BDPS)
Journal homepage: www.banglajol.info; www.bdpsjournal.org
Abstracted/indexed in Academic Search Complete, Agroforestry Abstracts, Asia Journals Online, Bangladesh Journals Online, Biological Abstracts, BIOSIS Previews, CAB Abstracts, Current Abstracts, Directory of Open Access Journals, EMBASE/Excerpta Medica, Global Health, Google Scholar, HINARI (WHO), International Pharmaceutical Abstracts, Open J-gate, Science Citation Index Expanded, SCOPUS and Social Sciences Citation Index
ISSN: 1991-0088; DOI: 10.3329/bjp.v21i1.87252

Bangladesh J Pharmacol 2026; 21: 35-38

Letter to the Editor

Neuroprotective effect of juglone against 6-OHDA-induced neurotoxicity in PC12 cells

Dear Editor,

Parkinson's disease is one of the most common neurodegenerative diseases, predominantly affecting middle-aged and elderly individuals. Existing therapies cannot effectively slow Parkinson's disease progression (Tolosa et al., 2021). Therefore, it is necessary to put further effort in developing new drugs that can effectively prevent or treat PD.

In modern medicine, many new drugs originate from the utilization of natural products (Newman et al., 2020). Paclitaxel, originally isolated from the bark of Pacific yew (*Taxus brevifolia*), is a key chemotherapy drug for treating breast and ovarian cancers. Vinblastine and vinorelbine are the potent anticancer drugs derived from Madagascar periwinkle (*Catharanthus roseus*) (Cragg et al., 2016). Galantamine is obtained from snowdrops (*Galanthus spp.*), which alleviates symptoms of Alzheimer's disease (Li et al., 2025).

Juglone (5-hydroxy-1,4-naphthoquinone), a naturally-occurring naphthoquinone compound, is extracted primarily from walnut trees, including Manchurian walnut (*Juglans mandshurica*), black walnut (*J. nigra*), walnut (*J. regia*) and butternut (*J. cinerea*). *J. mandshurica* Maxim is one of the most important medical plants (Figure 1A), and have reported that its juglone possessed anti-cancer, antihypertensive, anti-diabetic, anti-inflammatory and immunomodulatory activities (Tang et al., 2022). However, the neuroprotective effects of juglone have been seldomly investigated. A recent study demonstrated that juglone could promote spinal cord injury recovery (Chen et al., 2025), indicating juglone might possess neuroprotective activity. Notably, juglone possesses excellent blood-brain barrier permeability property (Osztie et al., 2024). Therefore, this study aims to evaluate the neuroprotective effects of juglone against Parkinson's disease.

The neuroprotective properties of juglone (Must Biotechnology Co., Ltd., China) against Parkinson's disease were investigated using a 6-OHDA-injured PC12 cell model. Based on the MTT assay (Figure 1B), PC12 cells treated with 500 µM 6-OHDA for 12 hours was established as the model condition for subsequent studies, as this treatment significantly reduced cell viability by approximately 50%. Subsequently, the cytotoxicity of juglone on PC12 cells was assessed, revealing no significant toxicity within the 0.05–0.5 µM concentration range (Figure 1C). Next, the protective capacity of juglone to restore viability in 6-OHDA-damaged PC12 cells was evaluated. In the experimental design, PC12 cells were pretreated with juglone (0.05–2 µM) for 12 hours prior to 6-OHDA exposure. Subsequently, juglone was removed, and 6-OHDA was applied to induce neurotoxicity. Results demonstrated that pretreatment with juglone (0.05, 0.1, 0.2 and 0.5 µM) significantly reversed 6-OHDA-induced neuronal cell death (Figure 1D), suggesting that juglone exhibited good neuroprotective effects to protect against 6-OHDA-induced neurotoxicity in PC12 cells.

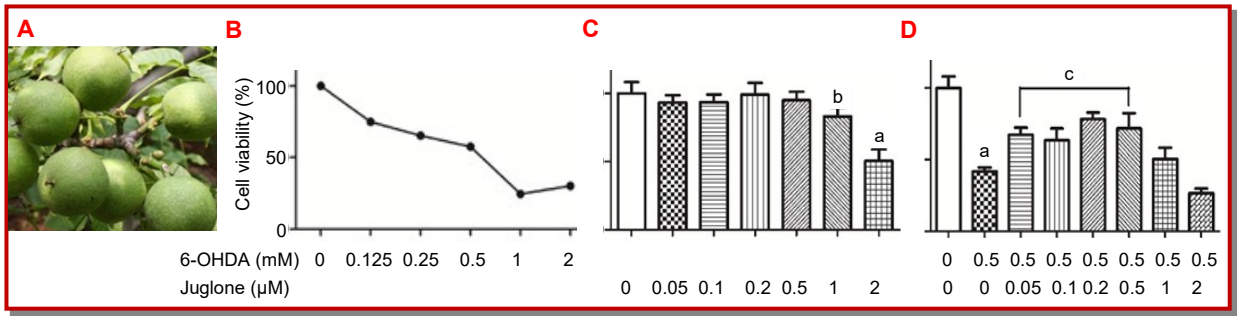


Figure 1: Effect of juglone on cell viability in 6-OHDA-injured PC12 cells. (A) The picture of *J. mandshurica;* (B) The effect of 6-OHDA on cell viability in PC12 cells. (C) The effect of juglone on cell viability in PC12 cells. (D) The neuroprotective effect of juglone against 6-OHDA induced neurotoxicity in PC12 cells. PC12 cells were pretreated with juglone (0.05-2 µM) for 12 hours prior to 500 µM 6-OHDA exposure for another 12 hours to induce neurotoxicity. The values are expressed as mean ± SEM of 6 determinations. Compared with the control group, $^{a}p<0.001$, $^{b}p<0.01$; compared with the 6-OHDA model group, $^{c}p<0.001$

Oxidative stress is widely recognized as a key factor in the degeneration of dopaminergic neurons within the pathogenesis of Parkinson's disease, characterized by excessive accumulation of nitric oxide and reactive oxygen species (ROS) (Andersen, 2004; Lin and Beal, 2006). Therefore, to investigate the protective mechanisms of juglone against 6-OHDA-induced damage in PC12 cells, this study employed DAF-FM DA probe staining to detect nitric oxide production levels and DCFH-DA probe staining to assess ROS generation levels in PC12 cells. Under fluorescence microscopy, green fluorescent puncta in PC12 cells represent nitric oxide or ROS signals. Compared with the control group, the 6-OHDA-treated cells displayed significantly enhanced green fluorescence, indicating excessive nitric oxide and ROS production in PC12 cells (Figure 2). When cells were pretreated with 0.05 and 0.1 μM juglone, the 6-OHDA-induced accumulation of nitric oxide and ROS was markedly reversed (Figure 2). These results indicated that juglone exerted neuroprotective effects by reducing excessive nitric oxide and ROS accumulation and alleviating cellular oxidative stress.

Mitochondria are highly vulnerable to oxidative stress, and mitochondrial damage triggers mitochondrial-dependent apoptosis in neurons, which serves as key contributor in the degeneration and death of dopaminergic neurons in Parkinson's disease (Kung et al., 2021). Mitochondrial membrane potential depolarization reflects mitochondrial damage and serve as an early apoptosis marker. This study employed JC-1 dye staining to evaluate the protective impact of juglone on mitochondrial membrane potential in 6-OHDA-induced damage in PC12 cells. JC-1 is a lipophilic cationic dye that is able to permeate through the mitochondrial membrane. In healthy cells with high mitochondrial membrane potential, JC-1 exists as aggregates and yields red fluorescence; whereas in depolarized mitochondria, JC-1 exists as monomers and yields green fluorescence. Thus, changes in mitochondrial membrane potential can be determined by measuring the

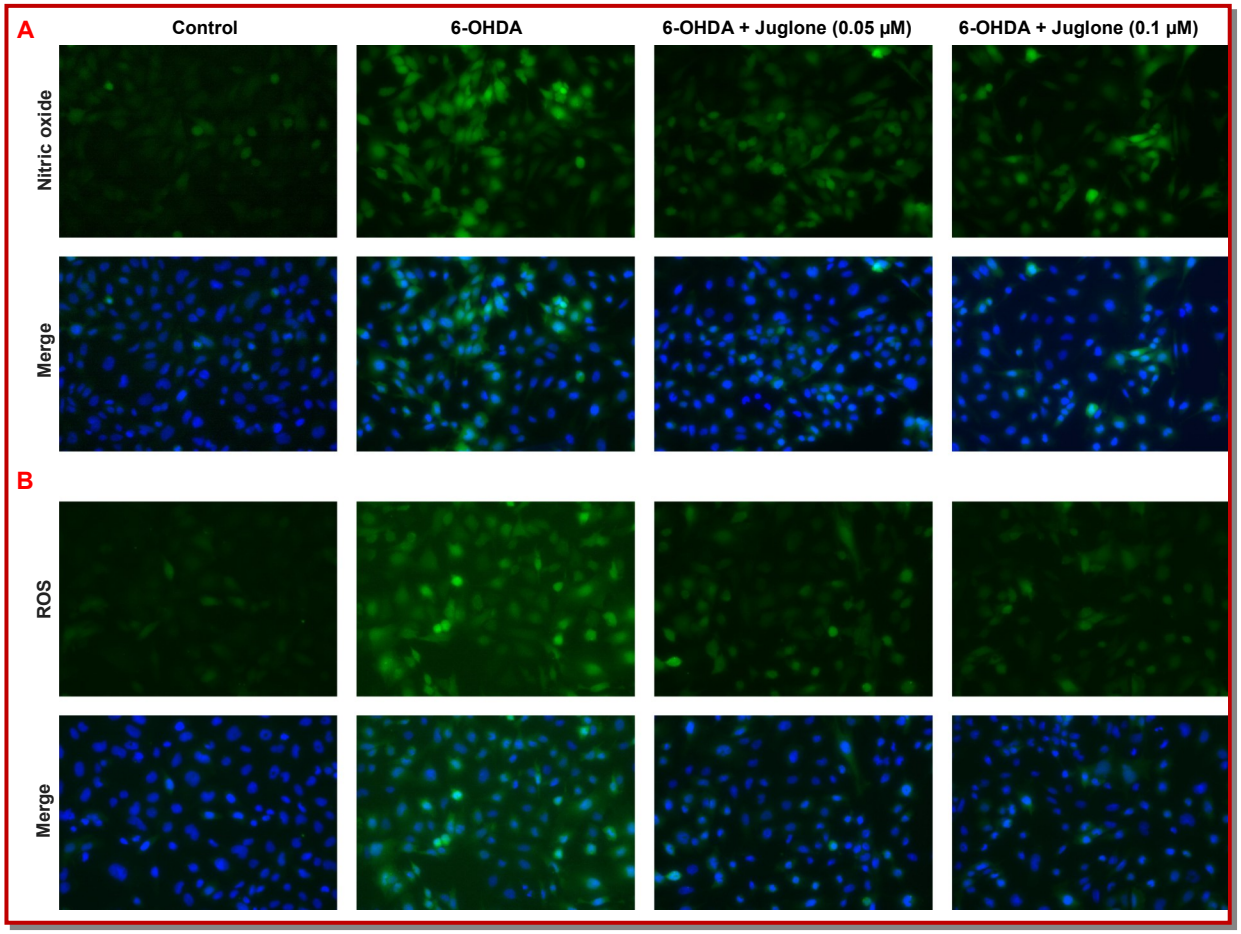


Figure 2: Effect of juglone on oxidative stress in 6-OHDA-injured PC12 cells. (A) DAF-FM DA probe staining demonstrated the protective effect of juglone against 6-OHDA-induced excessive nitric oxide production. The green fluorescence represented the signal of nitric oxide production, while the blue fluorescence indicated the signal of cell nuclei. (B) DCFH-DA probe staining demonstrated the protective effect of juglone against 6-OHDA-induced excessive ROS generation. The green fluorescence represented the signal of ROS generation, while the blue fluorescence indicated the signal of cell nuclei. The merged image showed the overlay of dual-channel signals from green and blue fluorescence. Images acquired by fluorescence microscope (200x)

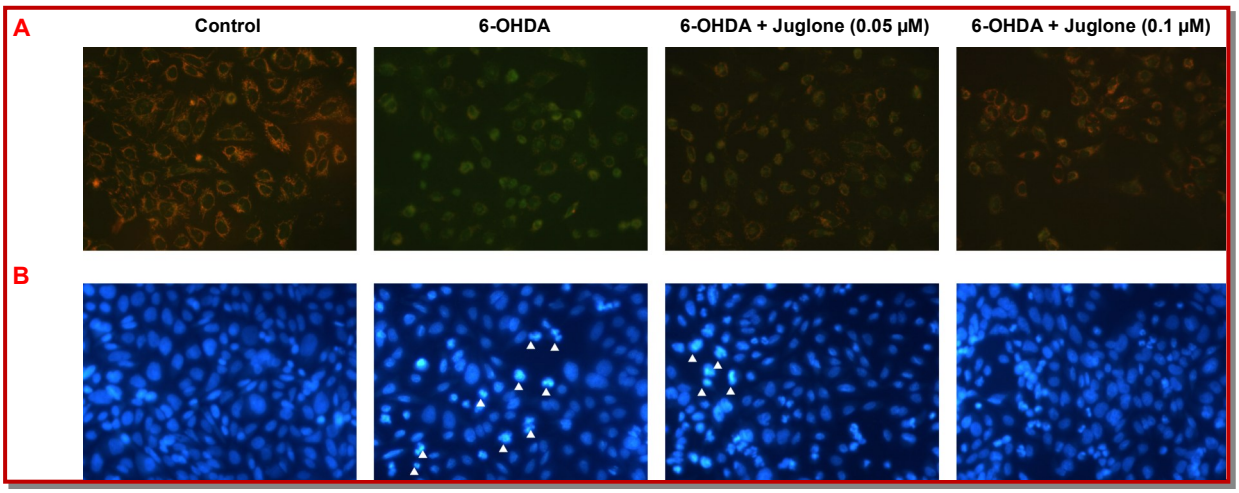


Figure 3: Effect of juglone on mitochondrial membrane potential loss and number of apoptotic bodies in 6-OHDA-injured PC12 cells. (A) JC-1-stained PC12 cells demonstrated the protective effect of juglone against 6-OHDA-induced mitochondrial membrane potential loss. (B) Hoechst 33342-stained PC12 cells demonstrated the anti-apoptotic effect of juglone against 6-OHDA-induced neurotoxicity. White arrows indicate the apoptotic bodies within the cell nucleus. Images acquired by fluorescence microscope (200x)

green/red fluorescence ratio. As shown in Figure 3A, compared to the control group, 6-OHDA-treated cells exhibited reduced red fluorescence and enhanced green fluorescence, indicating a significant decrease in mitochondrial membrane potential in PC12 cells. Pretreatment with juglone reversed the 6-OHDA-induced decline in mitochondrial membrane potential (Figure 3A). These results indicated that juglone mitigated the mitochondrial damage caused by 6-OHDA to mitochondrial membrane potential in PC12 cells. Furthermore, the cell apoptosis was examined by Hoechst 33342 staining. Under fluorescence microscopy, stained nuclei exhibited blue fluorescence. In untreated control cells, nuclei appeared round or elliptical, with no observation of nuclear condensation or fragmentation (Figure 3B). Conversely, bright blue fluorescent nuclei were observed in 6-OHDA-treated PC12 cells (Figure 3B), which meant nuclear condensation and fragmentation were occurring, indicating the presence of apoptotic bodies (as indicated by white arrows). Pretreatment with juglone significantly reduced the number of apoptotic bodies, demonstrating that juglone effectively inhibited 6-OHDA-induced apoptosis in PC12 cells (Figure 3B). Collectively, these results suggested that juglone suppressed apoptosis by mitigating the damaging effects of 6-OHDA on the mitochondrial membrane potential of PC12 cells.

In conclusion, this study demonstrated the neuroprotective effect of juglone in 6-OHDA-induced Parkinson's disease cell model. Juglone pretreatment markedly inhibited 6-OHDA-induced oxidative stress, mitochondrial damage, and neuronal apoptosis in PC12 cells as evidenced by reducing nitric oxide and ROS accumulation, enhancing mitochondrial membrane potential and decreasing number of apoptotic bodies. However, this study has limitations in further exploring molecular mechanisms, including elucidating the upstream signaling pathways through which juglone modulates oxidative stress and apoptosis, as well as investigating whether other regulatory mechanisms exist.

Financial support: This work was supported by National Natural Science Foundation of China (No. 32560887, 82560768), Hainan Province Science and Technology Special Fund (No. ZDYF2025SHFZ022), Hainan University Collaborative Innovation Center Research Fund (No. XTCX2022JKB01) and Hainan Nanhai New Star Project (No. 202309009)

Ethical Issue: The development, acquisition, authentication, cryopreservation, and transfer of cell lines between laboratories were followed according to the guidelines published in British Journal of Cancer, 2014

Conflict of interest: The authors declare no competing interests

Acknowledgment: The authors thank members of the Ding laboratory for helpful advice and discussion

Qingqing Liu, Jiaolin Bao, and Ren-Bo Ding

Key Laboratory of Tropical Biological Resources of Ministry of Education, School of Pharmaceutical Sciences, Collaborative Innovation Center of Life and Health, Hainan University, Haikou, 570228, China.

Corresponding author: Ren-Bo Ding

Email: dingrenbo@hainanu.edu.cn

References

Andersen JK. Oxidative stress in neurodegeneration: Cause or consequence?. Nat Med. 2004; 10(Suppl 7): S18-25.

Chen L, Chang Y, Zhang S, Wang Z, Liu H, Wang H, Shi L, Li N, Feng S. Juglone promotes spinal cord injury recovery by suppressing pyroptosis and necroptosis through FOS/USP53/ubiquitination. Phytomedicine 2025: 148: 157341.

Cragg GM, Pezzuto JM. Natural products as a vital source for the discovery of cancer chemotherapeutic and chemopreventive agents. Med Princ Pract. 2016; 25: 41-59.

Kung HC, Lin KJ, Kung CT, Lin TK. Oxidative stress, mitochondrial dysfunction, and neuroprotection of polyphenols with respect to resveratrol in Parkinson's disease. Biomedicines 2021; 9: 918.

Li ZH, Zhang ZQ, Yu B. Unlocking the therapeutic potential of natural products for Alzheimer's disease. J Med Chem. 2025; 68: 2377-402.

Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 2006; 443: 787-95.

Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. J Nat Prod. 2020; 83: 770-803.

Osztie R, Czegledi T, Ross S, Stipsicz B, Kalydi E, Beni S, Boldizsar I, Riethmueller E, Bosze SE, Alberti A. Comprehensive characterization of phytochemical composition, membrane permeability, and antiproliferative activity of *Juglans nigra* polyphenols. Int J Mol Sci, 2024; 25: 6930.

Tang YT, Li Y, Chu P, Ma XD, Tang ZY, Sun ZL. Molecular biological mechanism of action in cancer therapies: Juglone and its derivatives, the future of development. Biomed Pharmacother. 2022; 148: 112785.

Tolosa E, Garrido A, Scholz SW, Poewe W. Challenges in the diagnosis of Parkinson's disease. Lancet Neurol. 2021; 20: 385-97.