Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **36** (8): 1631-41 (2017)

Received: March 4, 2017
Revised version: June 6, 2017
Accepted: June 9, 2017

Osthole Prevents 6-Hydroxydopamine (6-OHDA) Induced Endoplasmic Reticulum Stress and Cytotoxicity Through Activating Akt/GSK3beta Signaling Pathway in PC12 Cells

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***SUMMARY*. Osthole, a coumarin compound extracted from *Cnidium monnieri,* was found potently attenuated 6-hydroxydopamine (6-OHDA)-induced cytotoxicity and apoptosis. It significantly decreased intracellular reactive oxygen species (ROS) and prevented the decline of mitochondrial membrane potential and intracellular GSH level induced by 6-OHDA. As reported, 6-OHDA evoked endoplasmic reticulum (ER) stress, which plays a major role in several neurodegenerative diseases, including Parkinson's disease (PD). Quite interestingly, osthole markedly reduced 6-OHDA induced ER stress, including the upregulated levels of glucose-regulated protein-78 (GRP78), and C/EBP homologous protein (CHOP) expression. Moreover, treatment with osthole restored the p-Akt and p-GSK-3β (Ser9) reduced by 6-OHDA. Furthermore, the protective effect of osthole on ER stress was attenuated by LY294002, a selective PI3K inhibitor. The shRNA knockdown results showed that down regulated Akt1 or GSK-3β attenuated the protective effect of osthole, indicating that the Akt/GSK-3β pathway was involved in the neuroprotective effect of osthole. Taken together, our findings suggest that the protective effect of osthole against 6-OHDA-induced ER stress and oxidative stress injuries in PC12 cells involves the Akt/GSK-3β pathway.**

RESUMEN. El osthol, un compuesto cumarínico extraído de *Cnidium monnieri,* se encontró que atenuaba potentemente la citotoxicidad y apoptosis producida por la 6-hydroxydopamina (6-OHDA). Redujo significativamente las especies de oxígeno reactivo intracelular (ROS) y evitó la disminución del potencial de membrana mitocondrial y el nivel de GSH intracelular inducido por 6-OHDA. Como se informó, 6-OHDA evocó el estrés endoplasmático (ER), que desempeña un papel importante en varias enfermedades neurodegenerativas, incluida la enfermedad de Parkinson (PD). De manera bastante interesante, el osthol redujo notablemente el estrés de la ER inducida por 6-OHDA, incluyendo los niveles regulados por la glucosa de la proteína-78 (GRP78) regulada por la glucosa y la expresión de la proteína homóloga C/EBP (CHOP). Por otra parte, el tratamiento con osthol restauró los valores del p-Akt y p-GSK-3β (Ser9) reducido por 6-OHDA. Además, el efecto protector del osthol sobre el estrés de ER se atenuó por LY294002, un inhibidor selectivo de PI3K. Los resultados de knockdown del shRNA mostraron que la regulación de Akt1 o GSK-3β atenúa el efecto protector de osthole, lo que indica que la vía Akt/GSK-3 participó en el efecto neuroprotector de osthol. Tomados en conjunto, nuestros hallazgos sugieren que el efecto protector del osthol contra el estrés de ER inducido por 6-OHDA y las lesiones por estrés oxidativo en células PC12 implican la vía Akt/GSK-3β.

KEY WORDS: apoptosis, endoplasmic reticulum stress, GSK-3beta, 6-OHDA, osthole, Parkinson's disease.

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ISSN 0326 2383 (printed ed.)
ISSN 2362-3853 (on line ed.)