Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **38** (9): 1894-1903 (2019)

Received: June 2, 2019
Revised version: June 12, 2019
Accepted: June 15, 2019

6'-O-β-D-Glucopyranosyl-12a-hydroxydalpanol Downregulates NO and PGE_2 Production in BV2 Microglia Cells by Suppressing PI3K/Akt-Mediated NF-κB Pathway

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SUMMARY. **We examined the anti-inflammatory effect of 6'-O-β-D-glucopyranosyl-12a-hydroxydalpanol (GHD) isolated from *Amorpha fruticosa* L. in lipopolysaccharide (LPS)-stimulated BV2 microglial cells. GHD was extracted from dried and powdered *A. fruticosa* fruits using methanol and dichloromethane along with sephadex gel filtration and silicagel chromatography, and characterized the chemical structure using nuclear magnetic resonance spectroscopy and mass spectrometry. GHD treatment did not showed cytotoxicity at 150 μM in BV2 microglial cells, in either the presence or absence of LPS. Additionally, pre-treatment with GHD significantly inhibited the excessive LPS-induced production of nitric oxide (NO) and prostaglandin E_2 (PGE_2). This inhibition was shown to be associated with the downregulation of inducible NO synthase (*iNOS*) and cyclooxygenase-2 (*COX-2*) mRNA and protein levels. The mechanism underlying the GHD-mediated attenuation of LPS-induced *iNOS* and *COX-2* expression involved retaining nuclear factor-κB (NF-κB) subunits, p65 and p50, within the cytosolic compartment, as well as inhibiting the phosphorylation of inhibitor of κBα (IκBα), ultimately leading to a reduction in the DNA-binding activity of NF-κB. We also found that GHD suppressed the generation of proinflammatory mediators such as NO and PGE_2, as well as their associated genes, *iNOS* and *COX-2*, in LPS-stimulated BV2 microglial cells, by inhibiting the PI3K/Akt-mediated NF-κB signaling pathway. Taken together, these data indicate that GHD possesses great potential for treating LPS-induced inflammatory responses.**

RESUMEN. Examinamos el efecto antiinflamatorio del 6'-O-β-D-glucopiranosil-12a-hidroxialpanol (GHD) aislado de *Amorpha fruticosa* L. en células microgliales estimuladas con lipopolisacárido (LPS) BV2. GHD se extrajo de frutos de *A. fruticosa* secos y en polvo utilizando metanol y diclorometano junto con filtración por gel de sephadex y cromatografía de gel de sílice, y se caracterizó la estructura química mediante espectroscopía de resonancia magnética nuclear y espectrometría de masas. El tratamiento con GHD no mostró citotoxicidad a 150 μM en células microgliales BV2, en presencia o ausencia de LPS. Además, el tratamiento previo con GHD inhibió significativamente la producción excesiva inducida por LPS de óxido nítrico (NO) y prostaglandina E_2 (PGE_2). Se demostró que esta inhibición está asociada con la regulación a la baja de los niveles de ARNm y proteínas de la NO sintasa (*iNOS*) y de la ciclooxigenasa-2 (*COX-2*) inducibles. El mecanismo subyacente a la atenuación mediada por GHD de la expresión de *iNOS* y *COX-2* inducida por LPS implicaba retener las subunidades del factor nuclear-κB (NF-κB), p65 y p50, dentro del compartimento citosólico, así como inhibir la fosforilación del inhibidor de κBα (IκBα), que finalmente conduce a una reducción en la actividad de unión al ADN de NF-κB. También encontramos que GHD suprimió la generación de mediadores proinflamatorios como NO y PGE_2, así como sus genes asociados, *iNOS* y *COX-2*, en células microgliales BV2 estimuladas con LPS, mediante la inhibición de la señalización de NF-κB mediada por PI3K/Akt. En conjunto, estos datos indican que la GHD posee un gran potencial para tratar las respuestas inflamatorias inducidas por LPS.

KEY WORDS: Amorpha fruticosa, 6'-O-β-D-Glucopyranosyl-12a-hydroxydalpanol, Nitric Oxide, Nuclear Factor-κB, Prostaglandin E2.

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