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Anti-Inflammatory Effects of Oleifoliosides A Isolated from *Dendropanax morbifera* Lev on LPS-Induced NO Production in RAW 264.7 Macrophage Cells

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Objectives

Dendropanax morbifera Leveille (Araliaceae) is an endemic species and used in folk medicine for the treatment of headache, infectious diseases, skin diseases and malady. To investigate the mechanisms of the anti-inflammation, we isolated Oleifoliosides A (OFSA) from *Dendropanax morbifera* Lev. and examined production of pro-inflammatory mediators such as nitric oxide (NO) and prostaglandin E_2 (PGE_2) in LPS-induced RAW 264.7 cells. We also assessed expressions of inducible nitric oxide synthase (iNOS) and cyclooxigenase-2 (COX-2), which are involved in immune and inflammatory responses, at the protein and mRNA level using Western blotting and RT-PCR, respectively. Because nucler transcription factor κB (NF-κB) is involved in the expression of inflammatory mediator gene, activation and translocation of NF-κB were determined by electrophoretic mobility shift assay (EMSA) and immunofluorescence staining using confocal microscopy, respectively.

Materials and Methods

- Isolation of Oleifoliosides A (OFSA) from *Dendropanax morbifera*
- Cell culture : RAW 264.7 macrophage cells
- Lipopolysaccharide (LPS): Sigma
- DAPI and FITC-conjugqted antibody
- MTT cytotoxicity assay
- NO production, PGE_2 and TNF-α ELISA assay
- RT-PCR and Western blot assay
- iNOS promoter assay, DAPI staining
- EMSA and immunocytochemical analysis

Results

OFSA significantly inhibited the LPS-induced NO and PGE_2 productions in LPS-

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induced RAW 264.7 macrophage cells. The decrease in quantity of NO product was accompanied by the decrease in the iNOS protein and its mRNA expression levels. In addition, the expression level of COX-2 protein responsible for PGE_2 production was inhibited by OFSA treatment in a dose-dependent manner. OFSA suppressed NF-κB DNA binding activity and nuclear translocation of p65, which was accompanied by inhibition of IκB-α phosphorylation and IκB-α degradation. These results suggest that inhibition of NO production by OFSA occurs via blocking the phosphorylation as well as the degradation of IκB-α protein, thus preventing the translocation and activation of NF-κB in the nucleus.

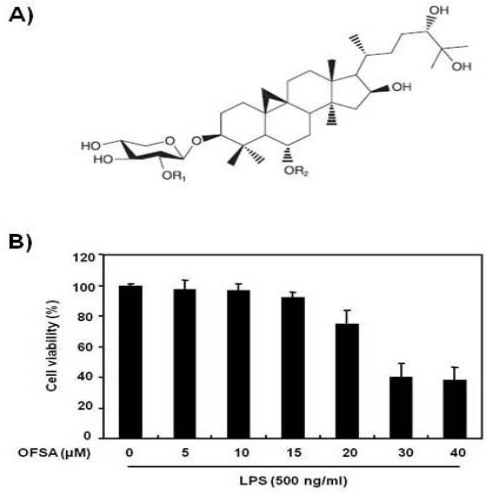

Fig. 1. Structure of Oleifoliosides A (OFSA) and effect of OFSA on cell viability in RAW 264.7 cells.

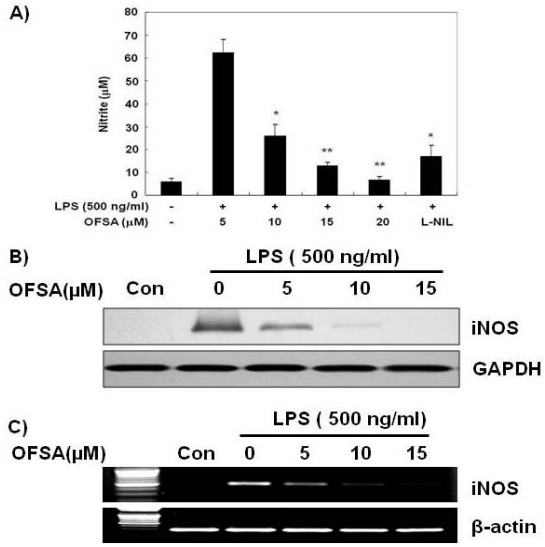

Fig. 2. Effects of OFSA on LPS-induced nitrc oxide (NO) production and inducible NO synthase (iNOS) expression in RAW 264.7 cells.