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Anti-Inflammatory Effect of Fermented *Viola patrinii* Extract

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Objectives

In an attempt to search for bioactive natural products exerting potent physiological activities, we prepared chloroform extract from the fermented *Viola patrinii* (Violaceae) (CEFV), and identified that CEFV showed significant effects on anti-inflammatory activity. CEFV inhibited 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) at the protein and mRNA level using Western blot and RT-PCR, respectively. Furthermore, translocation and activation of the nucler transcription factor κB (NF-κB) were determined by western blot analysis and electro mobility shift assay (EMSA), respectively.

Materials and Methods

○ Materials

- CH_3Cl extract from fermented *Viola patrinii* (CEFV)
- Cell line : RAW 264.7 macrophage cells
- Lipopolysaccharide (LPS): Sigma

○ Methods

- MTT cytotoxicity assay
- NO production assay
- PGE_2 and TNF-α ELISA assay
- RT-PCR and Western blot
- iNOS promoter assay

Results and Discussion

In the present study, CEFV inhibited the LPS-induced NO and PGE_2 productions in a dose-dependent manner in LPS-stimulated 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. CEFV also reduced the expression level of

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COX-2 protein responsible for PGE_2 production in LPS-stimulated RAW 264.7 macrophage cells. In addition, the LPS-induced DNA binding activity of NF-κB was significantly inhibited by CEFV, but AP-1 activation was not affected. In addition, CEFV suppressed LPS-induced phosphorylation of extracellular signal regulated kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK), but not p38 MAP kinase. These results suggest that CEFV has the inhibitory effects on LPS-induced NO and PGE_2 production and expression of iNOS and COX-2 in RAW 264.7 macrophage cells through blocking NF-κB activation via ERK1/2 and JNK activation.

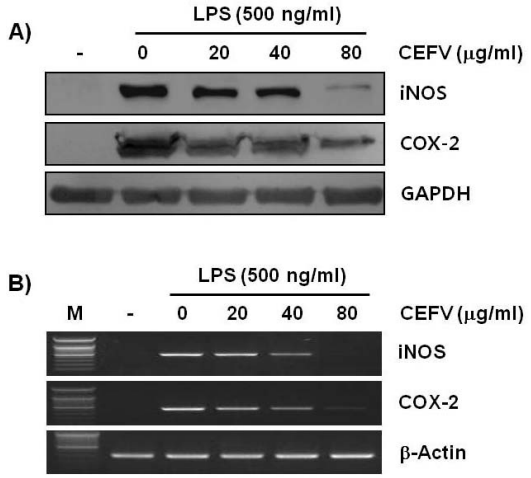

Fig. 1. Effects of chloroform extract from the fermented *V. patrinii* (CEFV) on LPS-induced iNOS and COX-2 protein and mRNA expression in RAW 264.7 cells.

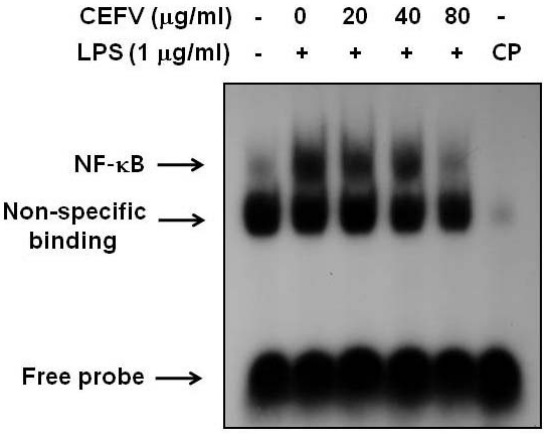

Fig. 2. Inhibition of NF-κB DNA binding activity by CEFV in LPS-induced RAW 264.7 cells