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Methaol extract of Theaceae supresses nitrite synthesis and expression of inflammatory cytokines

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Objectives

The purpose of this study is to elucidate effects of methanol extract R-518 from leaves of a *Theaceae* on production of inflammatory mediators and cytokines in macrophage cell line and *in vivo* effects in collagen-induced arthritis model mice.

Materials and Methods

Cell line: Murine macrophage cell line RAW264.7 (Korean Cell Line Bank) was maintained in DMEM (2 mM L-glutamine supplemented with 100 unit/ml of penicillin), 100 mg/ml of streptomycin and 10% fetal bovine serum.

NO synthesis and COXII activity: The presence of nitrite, a stable oxidized product of NO, was determined in cell culture media by Griess reagent from Sigma (St. Louis, MO). PGE_2 in the culture supernatant was measured by PGE_2 assay ELISA kit (Alexis, San Diego, CA).

Analysis of cytokine gene expression : mRNA expression levels were detected by RT-PCR analysis and protein levels were detected by Western blot analysis.

Results and Discussion

Theacea extracts regulated production of nitric oxide (NO) and prostaglandin E2 (PGE2) in vitro. The extract inhibited the mRNA and protein expression of inducible nitric oxide (iNOS) and cyclooxygenase-2 (COX-2) in LPS-stimulated RAW264.7 in dose dependant manner. *Theacea* extracts also inhibited the production of that inflammatory cytokine such as IL-1α, IL-1β, IL-6, TNF-α. The R-518 extract suppresses the IkB-a degradation and phosphorylation in LPS-stimulated RAW264.7 cells, indicating that the inhibition of the cytokine gene expression was mediated by NF-kB pathway. Moreover, administration of the R-518 extract to the collagen induced arthritis model mice demonstrated that R-518 markedly reduced arthritis symptom. These results suggest that R-518 may be beneficial for treatment of inflammatory diseases such as arthritis.

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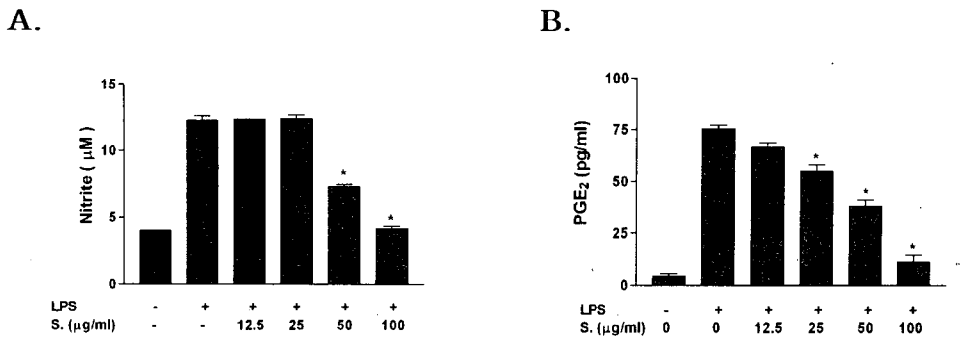

Fig. 1 Effect of *Theacea* extracts on NO production and PGE_2. RAW264.7 cells were treated with LPS (1 μg/ml) in the presence of various concentrations of *Theacea* extracts. A. Nitrite levels were measured in the culture media of LPS-stimulated cells for 24 hr by Griess reaction in manufacturer's instruction. Data shown are the mean values ± SD (n = 3). *, $p < 0.05$ versus LPS alone. B. PGE_2 concentration was measured in the 24 hr culture media using a commercial ELISA kit recommended by manufacturer's instruction. Data shown are the mean values ± SD (n = 3). *, $p < 0.05$ versus LPS alone.

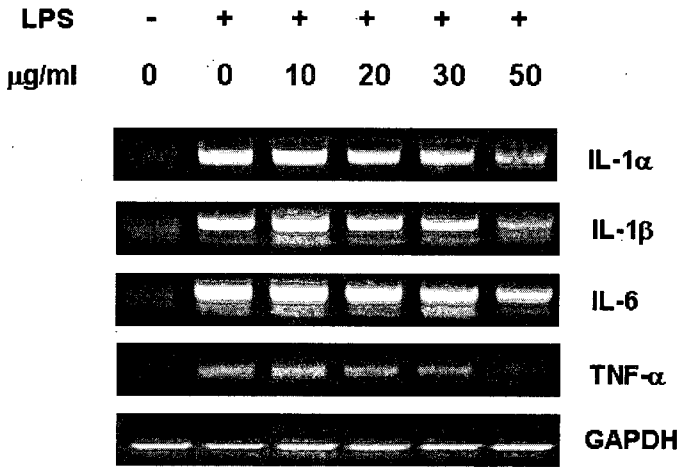

Fig. 2 Effect of *Theacea* extracts on the mRNA expression of pro-inflammatory cytokines in LPS-activated RAW264.7 cells. *Theacea* extracts suppressed on mRNA expression of IL-1a, IL-1b, IL-6 and TNF-a. RAW264.7 cells pre-incubated for 12 hr were incubated with *Theacea* extracts up to 100 mg/ml in the presence of LPS 1 μg/ml for 24 hr. The mRNA levels of IL-1α, IL-1β, IL-6 and TNF-α from the RAW264.7 cells were determined by RT-PCR.