Anti-inflammatory effect through Mitogen-activated protein kinases (MAPKs) signaling pathways of white ginseng extract (GS-KG9)

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Abstract

Background : This study is intended to evaluate the anti-inflammatory effect of white ginseng extract (GS-KG9) through the Mitogen-activated protein kinases (MAPKs) signaling pathway in the inflammatory response of RAW 264.7 cell induced by lipopolysaccharide (LPS).

Methods and Results : GS-KG9 was applied to LPS-induced RAW 264.7 macrophages. Nitric oxide (NO), prostaglandin E2 (PGE2) and pro-inflammatory cytokine interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) were determined using enzyme-linked immunosorbent analysis (ELISA). Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), mitogen-activated protein kinase (MAPK) subgroup extracellular signal ingested kinase (ERK), c-Jun N-terminal kinase (JNK) and P38 was detected by Western blot. GS-KG9 dose-dependently inhibited NO, PGE_2, and IL-6 production in LPS-stimulated RAW264.7 cells significantly. TNF-α was suppressed in high dose of GS-KG9. In addition, GS-KG9 attenuated LPS-induced overexpression of iNOS and COX-2 and suppressed the activation of ERK1/2, JNK, and p38.

Conclusion : These results indicate that GS-KG9 exerts anti-inflammatory effects via the inhibition of pro-inflammatory cytokines and down-regulating MAPKs signaling pathways.

Materials & methods

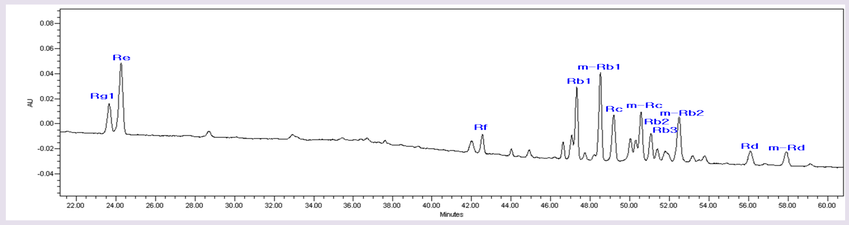
❖ Sample Information

Panax ginseng C. A. Meyer

❖ Preparation of GS-KG9

White ginseng
↓
Extraction
70% EtOH, 40℃ in vacuum
↓
Concentration
40℃ in vacuum
↓
Freeze dry
↓
GS-KG9

❖ HPLC chromatogram of GS-KG9



- **Characteristic compounds : Rg1, Rb1**
- **Criteria and Specifications : 12 ± 2.4 mg/g (Sum of Rg1 & Rb1)**

❖ Cell culture for anti-inflammatory assay

- Macrophage Raw 264.7 cells (5 × 10^4 cells/well)
- Dulbecco's Modified Eagle Medium (DMEM), FBS 10%, P/C 1%
- Lipopolysaccharide 1 μg/ml
- GS-KG9 : 25, 50, 100 μg/mL (in DMSO)

❖ Anti-inflammatory assay by ELISA

- MTT assay for cell viability
- Nitric oxide (NO)
- Prostaglandin E2 (PGE2)
- Interleukin -6 (IL-6)
- Tumor necrosis factor (TNF-α)

❖ Inflammatory protein by Western blot

- **Prostagrandine pathway**
  - Nitric oxide synthase (iNOS)
  - Cyclooxygenase-2 (COX-2)
- **MAPK signaling pathway**
  - ERK1/2, p- ERK1/2
  - JNK1/2, pJNK1/2
  - P38, pp38

Results

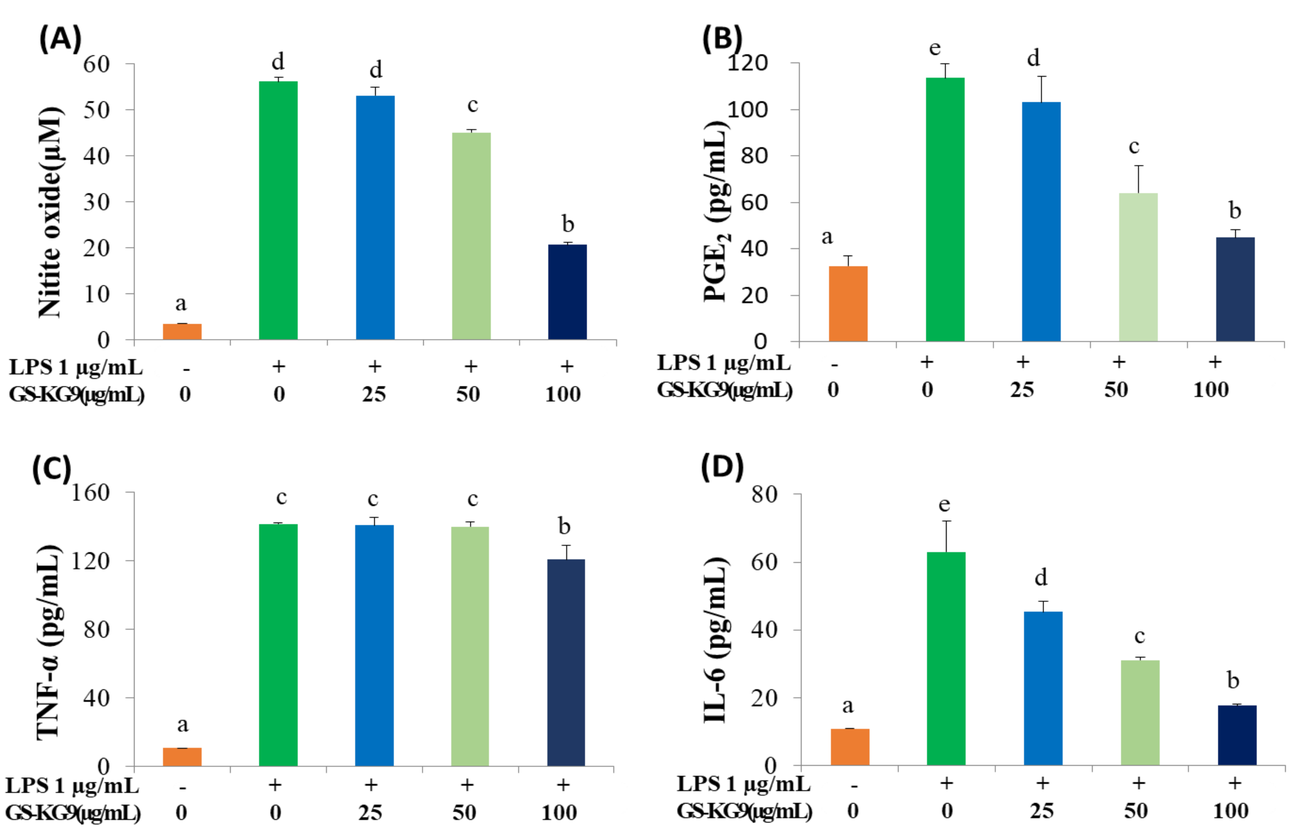


Fig. 1. Effect of white ginseng extract (GS-KG9) on nitric oxide (A), PGE_2 (B), TNF- α (C) and IL-6 (D) production by macrophage RAW 264.7 cells stimulated with 1 μg/ml lipopolysaccharide (LPS). The data represent the mean ± SD triplicate experiments.

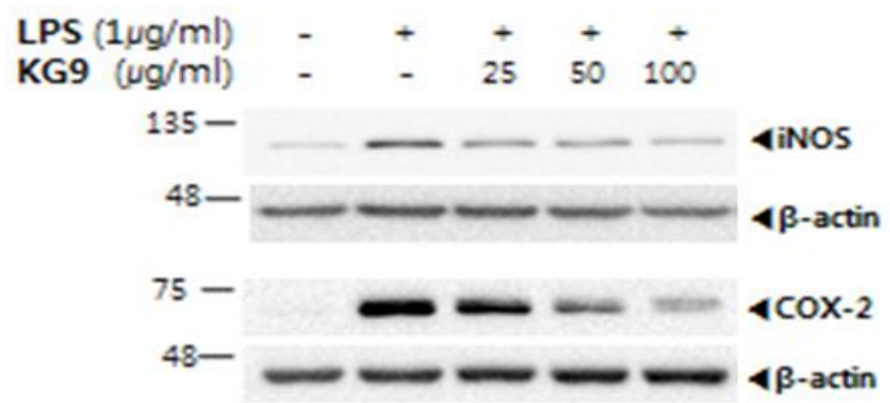


Fig. 2. Effect of GS-KG9 on the protein level of iNOS, COX-2 in LPS-stimulated RAW 264.7 cells. RAW 264.7 cells (5.0×10^4 cell/mL) stimulated with LPS (1 μg/mL) in the presence of GS-KG9 (25, 50 and 100 μg/mL) for 24 hr. Whole-cell lysates were prepared and the protein level was subjected to 10% SDS-PAGE, and expression of iNOS, COX-2 and β-actin were determined by western blotting. The β-actin as a loading control.

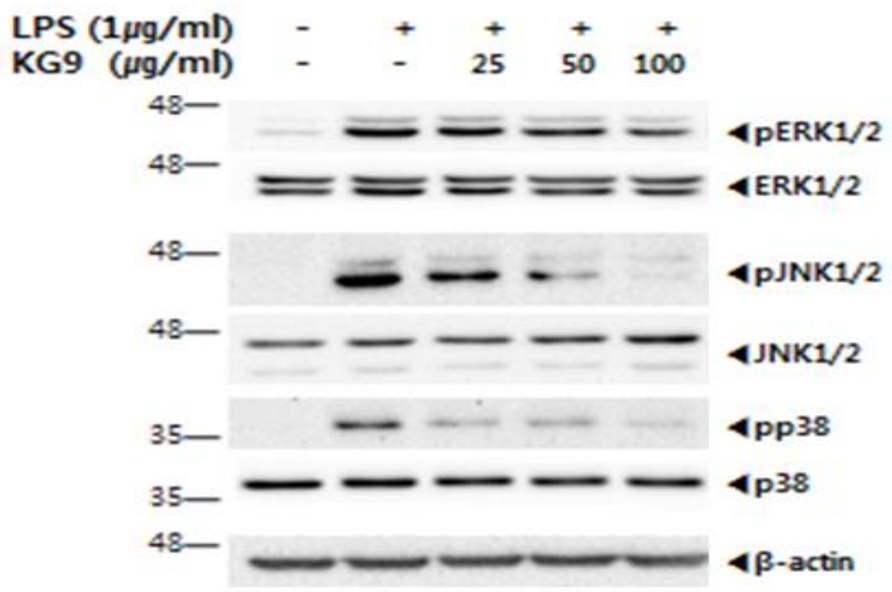


Fig. 3. Inhibitory effects of GS-KG9 on the protein expression of ERK1/2, p-ERK1/2, p-JNK1/2, JNK1/2, p-p38, and p38 in RAW 264.7 cells. The cells were treated with concentrations of GS-KG9 (25, 50 and 100 μg/mL) and LPS (1 μg/mL) for 1 hr. After treatment, total and phosphorylated (p-) expression levels were evaluated using western blotting specific antibodies.

Conclusion

● **GS-KG9 exerts anti-inflammatory effects via the inhibition of pro-inflammatory cytokines and down-regulating MAPKs signaling pathways**

Acknowledgements

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