Effects of *Asplenium incisum* with Antibacterial, Anti-inflammatory, and Anti-osteoclastogenic Activities on Periodontal Disease

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INTRODUCTION

The purpose of the study was to investigate the inhibitory effects of *Asplenium incisum* (AI) on *Porphyromonas gingivalis* (*P. gingivalis*) growth, the production of nitric oxide (NO) and pro-inflammatory cytokines (tumor necrosis factor-α [TNF-α], interlukin-6 [IL-6]), and anti-osteoclastogenesis.

MATERIALS & METHODS

Extracts of *Asplenium incisum* (AI)

1. Antibacterial assay
2. Sustainability of antibacterial activity
3. ELISA :TNF-α, IL-6 production
4. NO production
5. TRAP staining & activity
6. Cell viability : CCK-8 assay

RESULTS - Antibacterial activities

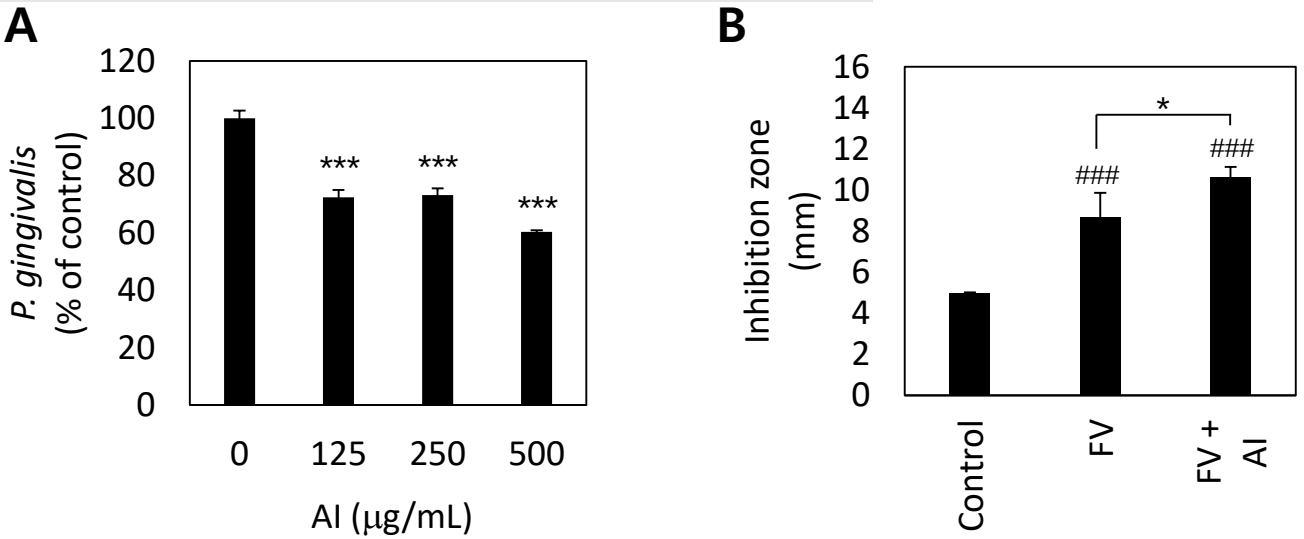

Figure 1. Inhibitory effect on bacterial growth and the sustainability of antibacterial activity of *Asplenium incisum* (AI). (A) The growth inhibitory effects of *P. gingivalis* according to the concentration of AI. (B) Sustained inhibitory effects against *P. gingivalis.*

The statistical analysis was performed by the student *t*-test. *** indicates significant differences from control (0 μg/mL AI) by the student *t*-test ($p<0.001$). ### means significant differences from the control (film disc) ($p<0.001$). * means significant differences between fluoride varnish (FV) and FV + AI groups ($p<0.05$).

RESULTS – Anti-inflammatory activities

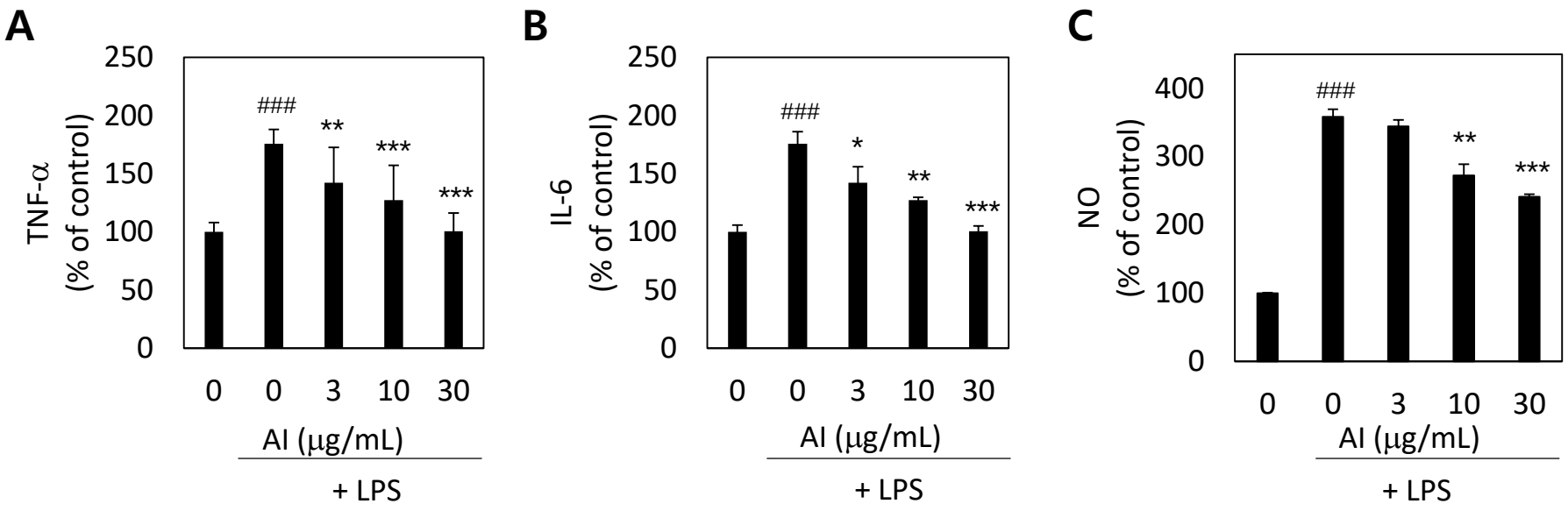

Figure 2. Effects of AI on TNF-α (A), IL-6 (B), and NO (C) production in lipopolysaccharide (LPS)-induced RAW 264.7 cells. The cells were pretreated with different concentrations of AI for 2 h and then exposed to 1 μg/mL LPS for 24 h. The levels of TNF-α, IL-6, and NO in the supernatant were measured at 540 nm by using a microplate reader. ### means the significant differences from the control group without LPS challenge (p<0.001). * indicates significant differences from the control (0 μg/mL AI) among the LPS treated groups (p<0.05), ** was (p<0.01) and *** was (p<0.001).

RESULTS – Anti-osteoclastogenic activities

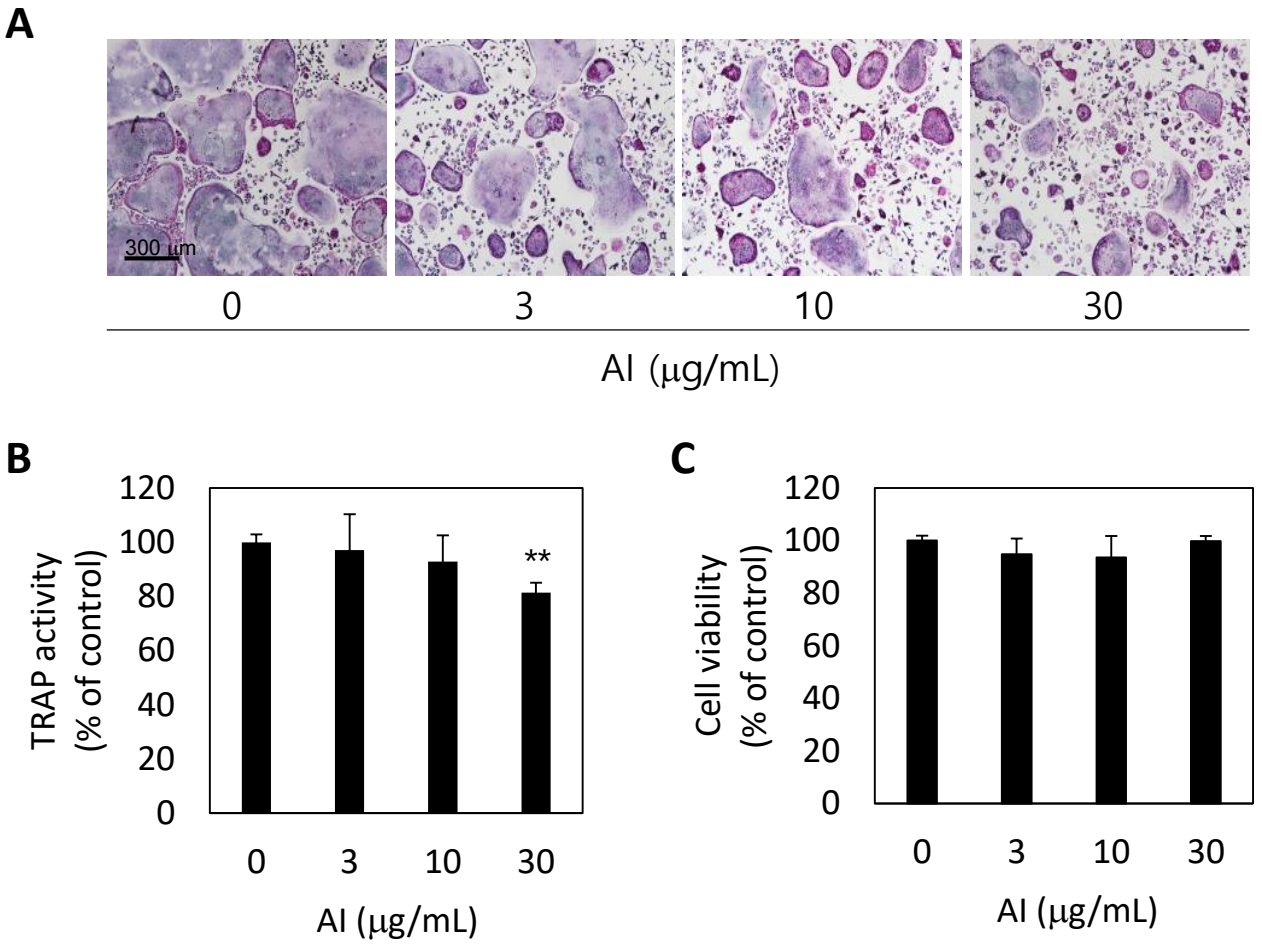

Figure 2. AI inhibits RANKL-induced osteoclastogenesis. BMMs were cultured for 4 days in the presence of macrophage colony-stimulating factor (M-CSF; 30 ng/mL) and receptor activator of nuclear factor-κB ligand (RANKL; 10 ng/mL) with 0, 3, 10, 30 μg/mL of AI. (A) Tartrate-resistant acid phosphate (TRAP) staining was performed to visualize osteoclast differentiation. Stained cells were photographed under a light microscope (magnification, 100). (B) TRAP activity was measured to evaluate the osteoclastogenic activity. (C) Cell viability was determined with the Cell Counting Kit-8 (CCK-8) assay. ** means the significant differences from the control group without AI treatment ($p<0.01$).

CONCLUSION

1. AI inhibited the growth of *P. gingivalis.*
2. FV+AI group showed significantly higher sustainability than did the FV group up to 3 days.
3. AI showed significant decreased of production of TNF-α, IL-6 and NO.
4. AI attenuated the formation of TRAP positive multinucleated osteoclasts and TRAP activity.
5. AI showed no cytotoxicity in BMMs.

Within the limitation of this study, AI was proven to have the potential to improve periodontitis through a combination of antibacterial, anti-inflammatory, and anti-osteoclastogenic activities.

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