

# An Aqueous Extract of Freeze-Dried *Protaetia brevitarsis* Larvae-induced Immunostimulation by Activating the NF- $\kappa$ B Signaling Pathway

Jayasingha J. A. C. C.<sup>1,2</sup>, Jayasooriya R. G. P. T.<sup>3</sup>, Choi Y. H.<sup>4</sup>, Kim G. Y.<sup>1,2</sup>✉

## Abstract

White-spotted flower chafer (*Protaetia brevitarsis*) larvae are a potential nutritional supplement and have been used in traditional Asian herbal medicine. In this study, we found that an aqueous extract of freeze-dried *P. brevitarsis* larvae (AEPB) promotes immunostimulation in RAW 264.7 macrophages. No significant cytotoxicity was observed below 800  $\mu$ g/mL AEPB. Moreover, AEPB treatment enhanced the production of nitric oxide (NO), prostaglandin E2 (PGE2), interleukin (IL)-6, and IL-12 through the upregulation of their regulatory genes. AEPB also promoted the nuclear translocation of nuclear factor- $\kappa$ B (NF- $\kappa$ B), and pyrrolidine dithiocarbamate, an inhibitor of NF- $\kappa$ B activation, remarkably prevented the expression of AEPB-induced inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2), IL-6, and IL-12, indicating that AEPB promotes the production of immunostimulants such as NO and PGE2 and pro-inflammatory cytokines such as IL-6 and IL-12 in RAW 264.7 macrophages by activating the NF- $\kappa$ B signaling pathway. Moreover, AEPB upregulated the extracellular expression of Toll-like receptor 4 (TLR4) and subsequently increased myeloid differentiation primary response 88 (MyD88) and IL-1 receptor-associated kinase 4 (IRAK4) expression, which indicates that AEPB activated the NF- $\kappa$ B signaling pathway through the TLR4-mediated MyD88 and IRAK4 axis. Collectively, this study provides evidence that AEPB is a promising nutritional supplement for stimulating macrophage-mediated immune responses.

**Keywords:** *Protaetia brevitarsis*, nuclear factor- $\kappa$ B, toll-like receptor 4, immunostimulation

---

<sup>1</sup> Department of Marine Life Science, Jeju National University, Jeju 63243, Republic of Korea

<sup>2</sup> Research Institute for Basic Sciences, Jeju National University, Jeju 63243, Republic of Korea

<sup>3</sup> Department of Bioprocessing Technology, Faculty of Technology, Rajarata University of Sri Lanka, Mihintale 50300, Sri Lanka

<sup>4</sup> Department of Biochemistry, College of Oriental Medicine, Dong-Eui University, Busan 47227, Republic of Korea

✉ Correspondence: [immunkim@jejunu.ac.kr](mailto:immunkim@jejunu.ac.kr)