Anthocyanins from *Hibiscus syriacus* L. Attenuate LPS-Induced Inflammation by Inhibiting the TLR4-Mediated NF-κB Signaling Pathway

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Excessive or chronic inflammation contributes to the pathogenesis of many inflammatory diseases such as sepsis, rheumatoid arthritis, and ulcerative colitis. *Hibiscus syriacus* L. has been used as a medicinal plant in many Asian countries, even though its anti-inflammatory activity has been unclear. Therefore, we investigated the anti-inflammatory effect of anthocyanin fractions from the *H. syriacus* L. varieties Pulsae (PS) on the lipopolysaccharide (LPS)-induced expression of proinflammatory mediators and cytokines in RAW264.7 macrophages. PS suppressed LPS-induced nitric oxide (NO) and prostaglandin E₂ (PGE₂) secretion concomitant with downregulation of inducible NO synthase (iNOS) and cyclooxygenase-2 (COX-2) expression. Furthermore, PS inhibited the production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and IL-12 in LPS-stimulated RAW264.7 macrophages. Further study showed that PS significantly decreased LPS-induced nuclear translocation of the nuclear factor-κB (NF-κB) subunits, p65 and p50. Molecular docking data showed that many anthocyanins from PS fit into the hydrophobic pocket of MD2 and bound to Toll-like receptor 4 (TLR4), indicating that PS inhibits the TLR4-MD2-mediated inflammatory signaling pathway. Especially, apigenin-7-O-glucoside most powerfully bound to MD2 and TLR4 through LYS122, LYS122, and SER127 at a distance of 2.205 Å, 3.098 Å, and 2.844 Å and SER441 at a distance of 2.873 Å (docking score: -8.4) through hydrogen bonding, respectively. Additionally, PS inhibited LPS-induced TLR4 dimerization/expression on the cell surface, which consequently decreased MyD88 recruitment and IRAK4 phosphorylation. PS completely blocked LPS-mediated mortality in zebrafish larvae by diminishing the recruitment of neutrophil and macrophages accompanied by low levels of proinflammatory cytokines. Taken together, our results indicate that PS attenuates LPS-mediated inflammation in both *in vitro* and *in vivo* by blocking the TLR4/MD2-MyD88/IRAK4-NF-κB axis. Therefore, PS might be used as a novel modulatory candidate for effective treatment of LPS-mediated inflammatory diseases.

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