ABSTRACT

This study was sought to explore the mechanism of anti-inflammatory effect of triphala in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages and in adjuvant-induced arthritic rats. In LPS stimulated RAW 264.7 cells, triphala (100-300μg/ml) significantly suppressed the production of inflammatory mediators (e.g., TNF-α, IL-1β, IL-6, MCP-1, VEGF, NO, and PGE2), intracellular free radicals, and release of lysosomal enzymes (e.g., acid phosphatase, β-galactosidase, N-acetyl glucosamindase, and cathepsin D) in a dose-related manner. With triphala, mRNA levels of genes for pro-inflammatory TNF-α, IL-1β, IL-6, and MCP-1, inflammatory iNOS and COX-2 enzymes, and NFκBp65 were down-regulated in the stimulated cells; in contrast, there was up-regulation of heme oxygenase-1 (HO-1) expression. Western blot analyses revealed that triphala suppressed the protein expression of NFκBp65 and p-NFκBp65 in the stimulated cells, which subsequently reduced over-expression of TNF-α, IL-17, iNOS, and COX-2 in a manner similar to that observed with BAY 11-7082, an IκB kinase inhibitor. Immunofluorescence analysis revealed inhibition of p-NFκBp65 nuclear translocation and COX-2 protein expression caused by triphala. Consistent with these findings, the animal studies here confirmed that triphala (100mg/kg/b.wt) exhibited anti-inflammatory effect by reducing paw edema, lysosomal enzymes, protein bound carbohydrates, oxidative stress markers, and mRNA expression of pro-inflammatory cytokines, inflammatory marker enzymes (iNOS and COX-2), receptor activator of nuclear factor kappa-B ligand (RANKL), and transcription factors (NFκB p65 and AP-1) in the paw tissues of adjuvant-induced arthritic rats. The levels of bone collagen were found to increase with decreased urinary constituents (hydroxyproline and total glycosaminoglycans) in triphala treated arthritic rats. In addition, the immunohistochemistry and western blot analysis also revealed decreased expression of inflammatory mediators (e.g, IL-17, COX-2, and RANKL) via inhibition of NF-κB activation. Taken together, the results here demonstrate that triphala has potential anti-inflammatory applications in both in vivo and in vitro that could be used for the treatment of inflammatory disorders, including rheumatoid arthritis.