

Anemonin, from *Clematis crassifolia*, potent and selective inducible

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Abstract

The aim of this study was to examine the anti-inflammatory effects of aerial part of *Clematis crassifolia* Benth. (Ranunculaceae) based on an iNOS inhibition in lipopolysaccharide (LPS) activated macrophages. Bioassay-guided fractionation and purification led to the isolation of ibotanolide B (1), calceolarioside B (2), trans-caffeic acid (3), anemonin (4) and 3',4',5,7-tetrahydroxy-6-C-glucopyranosylflavone (5). Their structures were elucidated on the basis of spectroscopic analysis. All these compounds inhibited NO production, detected as nitrite, in activated macrophages except 5. Among them, anemonin (4) was the most potent. Analyses of reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting revealed that it decreased the expression of iNOS mRNA and protein in activated RAW 264.7 cells. In isolated rat thoracic aortic rings, anemonin prevented the vascular hyporeactivity to phenylephrine induced by LPS whereas it did not affect acetylcholine-induced endothelial NO-dependent relaxation, an index of endothelial NOS (eNOS) activity. These results indicated that the potential anti-inflammatory effect of anemonin, the naturally occurring selective iNOS inhibitor, may provide a rationale for the medical use of *Clematis crassifolia*.