DOCTORAL THESIS

A study on the anti-inflammatory activity and mechanism of action of herba siegesbeckiae (Xixiancao)

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ABSTRACT

Herba Siegesbeckiae (HS, *Xixiancao* in Chinese) is a commonly used traditional Chinese medicinal herb for treating inflammatory disorders such as arthritis and rheumatoid arthritis (RA). In ancient materia medica books, HS is recorded to be the aerial part of *Siegesbeckia pubescens* (SP) which is also the sole plant origin of HS in the 1963 edition of the Chinese Pharmacopeia (ChP). The aerial parts of *Siegesbeckia orientalis* (SO) and *Siegesbeckia glabrescens* (SG) have been included as two additional origins for HS in each edition of ChP since 1977. Likewise, the aerial parts of these three species are recorded as origins for HS in the Hong Kong Chinese Materia Medica Standards (HKCMMS). HS has been reported to exert anti-inflammatory effects by inhibiting the MAPKs and NF-κB pathways that are the components of Toll-like receptor 4 (TLR4) signaling. Until now, no chemical or pharmacological comparison among the three *Siegesbeckia* herbs has been conducted to answer the question whether the three herbs could all be used as HS origins. The role of TLR4 in the anti-inflammatory effect of HS has not been determined yet. This study aims to determine whether the aerial parts of SP, SO and SG can all be used as HS, and to explore the involvement of the TLR4 pathways in HS's anti-arthritic action.

To determine whether the three *Siegesbeckia* plants can all serve as the origins of HS, we compared their fingerprint chromatograms and inhibitory effects on inflammatory mediators. Chemical analyses showed that the three species have different profiles, although they have common peaks in their fingerprint chromatograms. Hierarchical cluster analysis (HCA) and principal component analysis (PCA) of the common peaks demonstrated that all samples of the three species tend to be species-dependently grouped and separated. Ten components contributing to the species discrimination were identified, of which 8 are long-chain fatty acids/esters, and 2 are darutoside and hythiemoside A. Inhibitory effects of the three species on NO production and IL-6 secretion in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophages are different, with SG being the most and SP the least potent. These chemical and bioactivity assays support the notion that the three *Siegesbeckia* species cannot be equally used as the plant origins of HS.

To investigate the involvement of TLR4 signaling in the anti-inflammatory effect of HS, we evaluated the anti-arthritic effects of an ethanolic extract of HS (HS for short, the dried aerial part of SO) in rats with collagen-induced arthritis (CIA), and investigated the involvement of TLR4 signaling in the effects of HS in

the CIA rats and RAW264.7 macrophages. Results showed that HS possesses anti-arthritic effects and has no observable adverse effects. *In vitro* and *in vivo* mechanistic studies reveal that HS's therapeutic effects is at least partially attributed to its inhibitory action on the IRAK4/MAPKs/AP-1, IRAK4/MAPKs/NF-κB, IRAK4/PI3K/NF-κB and TRAF3/TBK1/IRF3 pathways. We further found that HS inhibits LPS-TLR4 binding. To find out the compound responsible for inhibiting LPS-TLR4 binding, we first identified chemicals in HS by UHPLC/Q-TOF-MS analysis. Subsequent simulated computational and experimental studies showed that ursolic acid is one of the main active components. These studies demonstrated the anti-arthritic activity, the TLR4-signaling-related mechanism of action, and the active compound of HS.

This work provides a chemical and pharmacological basis for determining whether the three *Siegesbeckia* genus herbs SP, SO and SG can all serve as the origins of HS; and also provides pharmacological justifications for the clinical application of HS in treating inflammatory disorders.

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