



Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

The anti-inflammatory activities of *Ainsliaea fragrans* Champ. extract and its components in lipopolysaccharide-stimulated RAW264.7 macrophages through inhibition of NF- κ B pathway

Xin Chen^{1,a}, Jingshan Miao^{a,b,1}, Hao Wang^c, Fang Zhao^a, Jie Hua^a, Peng Gao^a, Yue Wang^e, Luyong Zhang^{a,d,*}, Ming Yan^{a,*}

^a Jiangsu Key Laboratory of Drug Screening, China Pharmaceutical University, Nanjing 210009, PR China

^b Key Laboratory of Acupuncture and Medicine Research of Ministry of Education, Nanjing University of Chinese Medicine, 138 Xianlin Rd, Nanjing 210023, PR China

^c Department of Natural Medicinal Chemistry, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, PR China

^d Key Laboratory of Drug Quality Control and Pharmacovigilance, China Pharmaceutical University, Ministry of Education, Nanjing 210009, PR China

^e School of Pharmacy, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, PR China

ARTICLE INFO

Article history:

Received 31 December 2014

Received in revised form

6 March 2015

Accepted 3 May 2015

Keywords:

Ainsliaea fragrans Champ.

Dicaffeoylquinic acid

Gynecological diseases

Nitric oxide

Inducible nitric oxide synthase

Nuclear factor- κ B pathway

ABSTRACT

Ethnopharmacological relevance: *Ainsliaea fragrans* Champ. (*A. fragrans*) is a traditional Chinese herbal that contains components like 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid. It exhibits anti-inflammatory activities which has been used for the treatment of gynecological diseases for many years in China. The aims of the present study were to investigate the anti-inflammatory activities of *A. fragrans* and elucidate the underlying mechanisms with regard to its molecular basis of action for the best component.

Materials and methods: The anti-inflammatory effects of *A. fragrans* were studied by using lipopolysaccharide (LPS)-stimulated activation of nitric oxide (NO) in mouse RAW264.7 macrophages. Expression of inducible NO synthase (iNOS) and pro-inflammatory cytokines, inhibitory κ B α (I κ B α) degradation and nuclear translocation of NF- κ B p65 were further investigated.

Results: The present study demonstrated that *A. fragrans* could suppress the production of NO in LPS-stimulated RAW264.7 macrophages. Further investigations showed *A. fragrans* could suppress iNOS expression. *A. fragrans* also inhibited the expression of tumor necrosis factor- α and interleukin-6. *A. fragrans* significantly decreased the degradation of I κ B α , reduced the level of nuclear translocation of p65. All these results suggested the inhibitory effects of *A. fragrans* on the production of inflammatory mediators through the inhibition of the NF- κ B activation pathway.

Conclusion: Our results indicated that *A. fragrans* inhibited inflammatory events and iNOS expression in LPS-stimulated RAW264.7 cells through the inactivation of NF- κ B pathway. This study gives scientific evidence that validate the use of *A. fragrans* in treatment of patients with gynecological diseases in clinical practice in traditional Chinese medicine.

© 2015 Published by Elsevier Ireland Ltd.

1. Introduction

Ainsliaea fragrans Champ. (*A. fragrans*), belongs to the Asteraceae family (*Ainsliaea* DC.), is a folk herbal medicine named

Abbreviations: LPS, lipopolysaccharide; NO, nitric oxide; iNOS, inducible nitric oxide synthase; TNF- α , tumor necrosis factor- α ; IL-6, interleukin-6; I κ B α , inhibitory κ B; NF- κ B, nuclear factor- κ B

* Corresponding authors at: Jiangsu Key Laboratory of Drug Screening, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, PR China. Tel./fax: +86 25 83271142.

E-mail addresses: lyzhang@cpu.edu.cn (L. Zhang),

brookming@163.com (M. Yan).

¹ These authors equally contributed to this work.

<http://dx.doi.org/10.1016/j.jep.2015.05.004>

0378-8741/© 2015 Published by Elsevier Ireland Ltd.

‘Xingxiang Tuerfeng’ in China and has long history of medicinal practice in south China. The pharmacology studies have focused on its anti-inflammatory effects in vitro. More than 20 compounds (Xing et al., 2006; Liu et al., 2007; Wang and Liu, 2007) have been found over the last 30 years from *A. fragrans* including triterpenoids, flavonoid, sesquiterpenes (Wang et al., 2009), and phenolic acids. The major bioactive constituents of *A. fragrans* are phenolic compounds (Zhang et al., 2006), including 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid.

Nowadays, the whole plant was applied as one of the key ingredients in preparations of ‘Xingxiang Tuerfeng tablet’ and ‘compound Xingxiang Tuerfeng granule’, which have been used for curing gynecological diseases like cervicitis, endometritis, and pelvic

inflammation with significant clinical effects (Chen and Wang, 2010; Cui et al., 2014). As a common over-the counter (OTC) medicine in China, these preparations have been the first-line treatment of gynecological diseases (Su et al., 2014).

Inflammation is a local, protective response of the immune system. Excessive inflammatory responses can be harmful, as in diseases such as rheumatoid arthritis, Alzheimer's disease and septic shock syndrome (Tracey, 2002). Lipopolysaccharide (LPS), a component of the cell wall of Gram-negative bacteria, stimulates macrophages to produce pro-inflammatory mediators such as tumor necrosis factor alpha (TNF- α), interleukin-6 (IL-6), and inducible nitric oxide synthase (iNOS), which trigger a cascade responsible for the inflammatory response.

Nitric oxide (NO) participates in various physiological processes such as regulation of inflammation, neurotransmission, mitochondrial functions and apoptosis (Tennyson and Lippard, 2011). NO is synthesized from L-arginine by three types of NO synthases (NOSs): iNOS, neuronal NOS (nNOS), and endothelial NOS (eNOS) (Bredt, 1999). A great number of studies have showed that iNOS is the main enzyme to catalyze NO production in inflammatory condition (Nagy et al., 2007). Numerous studies have shown that in inflammatory conditions, iNOS gene transcription is chiefly modulated by the transcription factor nuclear factor (NF)- κ B (Connelly et al., 2003).

The functions of NF- κ B are performed in homo- or heterodimeric forms of Rel family proteins such as RelA (p65), RelB, cRel, p50 and p52. NF- κ B is present in the cytoplasm in an inactive state, complexed with the inhibitory κ B (I κ B) including I κ B α , I κ B β , I κ B ϵ ,

p105 and p100 (Beg et al., 1992; Ghosh and Hayden, 2008). Extracellular stimuli and/or pro-inflammatory cytokines can trigger NF- κ B-activated pathways. These inducers can bind to cell surface receptors and activate the I κ B kinase (IKK) complex (Pennington et al., 2001). Activated IKK catalyzes I κ B phosphorylated, which is then ubiquitinated and proteasome-mediated degradation (Lee et al., 1998). After degraded, I κ B no longer binds with NF- κ B, and the free NF- κ B translocates into the nucleus where it induces the expression of multiple inflammatory genes, such as TNF- α and iNOS (Tian et al., 2005).

Although many studies have been previously reported about the medical effects of *A. fragrans*, the mechanism of the plant modulating molecular and cellular inflammatory responses has not been elucidated. In this study, we investigated the anti-inflammatory effects of *A. fragrans* Champ. Extract (AF-Ext) and its components 3,5-dicaffeoylquinic acid (AF-P1) and 4,5-dicaffeoylquinic acid (AF-P2) and the molecular targets in LPS-activated macrophages.

2. Materials and methods

2.1. Plant material

The whole herb of *A. fragrans* ('Xingxiang Tuerfeng') was collected from Wuyuan city, Jiangxi Province, PR China, in June 2005, and authenticated by Prof. Minjian Qing (Dept. of Pharmacognosy, China Pharmaceutical University, Nanjing, China). A voucher specimen (no. 20050701) was deposited in the herbarium of China Pharmaceutical University, Nanjing, China.

2.2. Chemicals and reagents

All analytical grade solvents used for column chromatography and HPLC grade methanol for HPLC analysis were purchased from Jiangsu Hanbang Science and Technology Co., Ltd. (Nanjing, China). Purified water was afforded by a Milli-Q system (Millipore, USA). HPD-100 resins were purchased from Cangzhou Bon Adsorber Technology Co., Ltd. (Hebei, China). Sephadex LH-20 (40–70 μ m) were purchased from Pharmacia Biotech AB (Uppsala, Sweden). Dimethyl sulfoxide (DMSO) and LPS from *Escherichia coli* 055: B5 were purchased from Sigma Chemical Co. (USA). NO detection kit was purchased from Beyotime Institute of Biotechnology (Haimen, China). Nuclear extract kit was purchased from Vazyme (Nanjing, China). Antibodies iNOS, I κ B α and NF- κ B p65 were purchased from Cell Signaling Technology Inc. (USA). Horseradish peroxidase (HRP)-conjugated goat anti-rabbit and goat

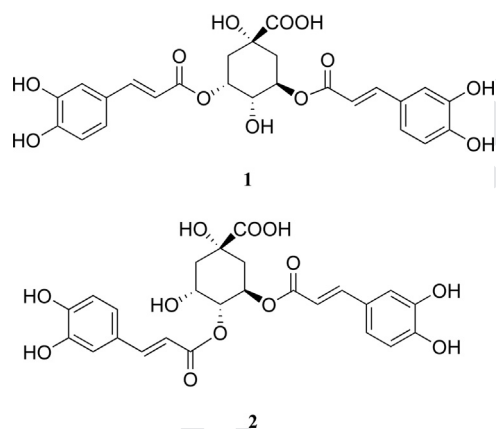


Fig. 1. Structures of compounds **1** and **2** isolated from *A. fragrans*.

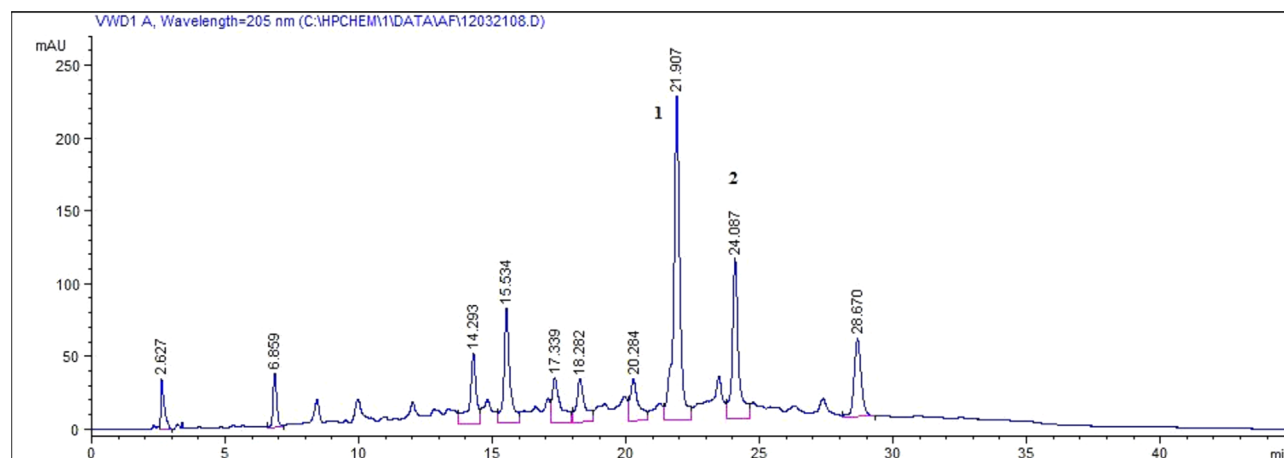


Fig. 2. HPLC Chromatogram of *A. fragrans* Extract.

Download English Version:

<https://daneshyari.com/en/article/5835104>

Download Persian Version:

<https://daneshyari.com/article/5835104>

[Daneshyari.com](https://daneshyari.com)