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Anti-inflammatory effects and mechanism of the total flavonoids from *Artemisia scoparia* Waldst. et kit. *in vitro* and *in vivo*

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ABSTRACT

Artemisia scoparia Waldst. et Kit. is traditionally used for the treatment of jaundice urinary retention, itching wet sores, infectious icteric hepatitis and influenza in Uighur medicine. This study aimed to further illuminate the anti-inflammatory effects and mechanism of the total flavonoids (ASTF) from Artemisia scoparia Waldst. et Kit. In vitro, RAW 264.7 cells were pretreated with ASTF 1 h before stimulation with LPS (1 μg/mL) for 24 h. Then, the concentrations of NO, PGE_2, TNF-α, IL-6 and MCP-1 in the medium were determined. Intracellular oxidative stress was detected using DCFH-DA. Immunofluorescent analysis, western blot and qRT-PCR were carried out to illuminate the mechanism of anti-inflammatory effects of ASTF. In vivo, mice were given an intragastric administration of ASTF 1 h before an intranasal administration of LPS. After 24 h, bronchoalveolar lavage fluid (BALF) was collected to measure the number of total cells, macrophage and neutrophils. The levels of TNF-α and IL-6 in BALF were quantified by ELISA kits. Lung specimens were isolated for histopathological examinations and lung wet-to-dry weight (W/D) ratio. We found that ASTF significantly inhibited the production of NO, PGE_2, TNF-α, IL-6, MCP-1 and reactive oxygen species (ROS) in LPS-stimulated RAW 264.7 cells. ASTF can obviously inhibit the degredation of IκBa and inhibit the nucleus translocations of p-NF-κB p65, p-ERK1/2 and p-p38 in RAW 264.7 cells stimulated by LPS. ASTF also markedly decreased the protein and mRNA expression of TNF-α and IL-6 in a dose-dependent manner. When pretreated with ASTF, alveolar hemorrhage and neutrophil infiltration, as well as pulmonary histopathologic changes, were substantially suppressed in lung tissues in the murine acute lung injury model. The lung wet-to-dry weight (W/D) ratio was strongly decreased. These results suggested that ASTF showed important anti-inflammatory activity and might provide protective effects against LPS-induced ALI. The anti-inflammatory effect of ASTF might attribute to its suppression of NF-κB and MAPK signaling pathway.

1. Introduction

Inflammation, as a response to tissue injury or infection, is vital for the body when facing invading microbes or diseases. Inflammatory response is mainly regulated by cytokines, eicosanoids, and nitric oxide (NO) released by the injured or infected cells and plays an important role in the progression of inflammation [1]. The proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL), are responsible for the communication of immune cells to promote the inflammatory process [2,3]. Macrophages also play an important role in the inflammation process. Lipopolysaccharide (LPS), a gram-negative bacterial endotoxin, stimulates macrophages to secrete chemokines such as CCL2/MCP-1 and CCL3/MIP1α, proinflammatory cytokines such as TNF-α, IL-6, and IL-1β [4], and inflammatory mediators to

Abbreviations: IL, interleukin; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; NO, nitric oxide; ROS, reactive oxygen species; PGE_2, prostaglandin E_2; MCP-1, monocyte chemotactic protein-1; iNOS, inducible form of nitric oxide synthase; COX, cyclooxygenase; IκB, inhibitor of κB binding protein; NF-κB, nuclear factor kappa B; MAPKs, mitogen-activated protein kinases; ERK, extracellular signal-regulated kinases; qRT-PCR, quantitative reverse transcriptase polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; MTT, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole; ALI, Acute lung injury; ARDS, acute respiratory distress syndrome; CMC-Na, sodium carboxymethyl cellulose

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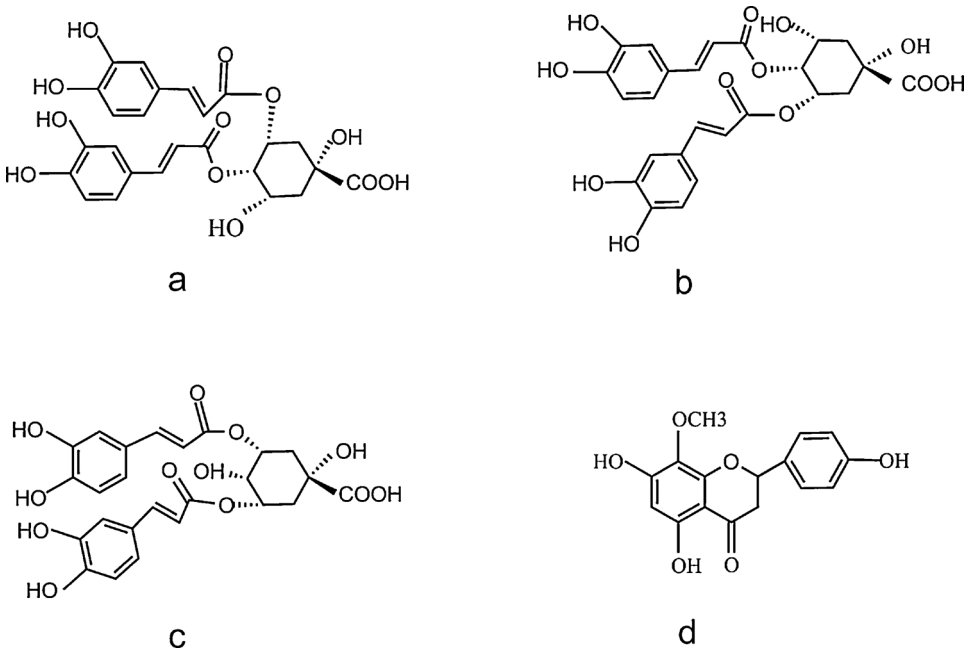

Fig. 1. Chemical Structure of Compounds. a. 4,5-di-O-caffeoylquinic acid; b. 3,4-di-O-caffeoylquinic acid; c. 3,5-di-O-caffeoylquinic acid; d. 4′-hydroxywogonin.

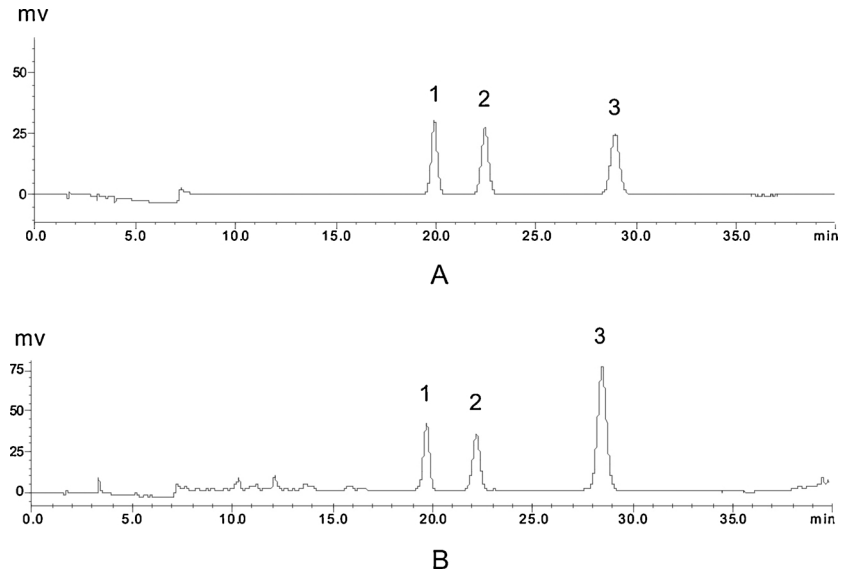

Fig. 2. HPLC chromatogram. A. reference substance; B. ASTF; 1. 3,4-di-O-caffeoylquinic acid; 2. 3,5-di-O-caffeoylquinic acid; 3. 4,5-di-O-caffeoylquinic acid.

amplify the host defense against the invasion from microbes [5–7]. Therefore, overproduction of these mediators will break immune homeostasis and cause several inflammatory diseases. It is well-known that murine macrophage RAW 264.7 cells stimulated by LPS, as an in vitro model, is often applied to the research on inflammatory effects and mechanisms of drugs.

Acute lung injury (ALI) is due to the uncontrolled and excessive production of inflammatory mediators. As the syndromes of acute respiratory failure, ALI and its more severe form, acute respiratory distress syndrome (ARDS), are characterized by intense pulmonary inflammatory responses, including neutrophil recruitment, disruption of epithelial integrity, interstitial edema, and lung parenchymal injury. ARDS often leads to multi-organ failure, with the mortality of approximately 30–50% [8]. LPS is one of the major factors inducing ALI [9]. The development of an ALI model using LPS instillation has become a basic investigation and therapeutic approach.

Artemisia scoparia Waldst. et Kit. belongs to composite plants of the genus Artemisia [10], which is called "xiwake" in Uighur and is used in traditional Uighur medicine for the treatment of jaundice urinary retention, itching wet sores, infectious icteric hepatitis and influenza [11]. Its main chemical compositions include flavonoids, chromogen ketone, coumarin. In our previous study, we optimized the extraction process of the total flavonoids (ASTF) from Artemisia scoparia Waldst. et Kit. We found that ASTF had the anti-inflammatory effect on auricle edema induced by xylene and increased permeability of blood capillary induced by acetic acid in mice, as well as relieve cough and act as an antipyretic [12]. ASTF could inhibit the production of IL-6, IL-8 and IFN-γin the lung tissue and serum in mice infected with PR8 influenza virus, and it also could improve pathological damage and decrease mortality rate and prolong survival[13–15].

In the present study, murine macrophage RAW 264.7 cells stimulated by LPS and acute lung injury induced by LPS in mice were adopted as in vitro and in vivo models, respectively, to further evaluate the anti-inflammatory effects and clarify the possible mechanisms of