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Protective effects of essential oils from *Rimulus cinnamon* on endotoxin poisoning mice

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ABSTRACT

The essential oils from *Rimulus cinnamon* (EORC) have anti-inflammation activities, but the effects of EORC on endotoxin poisoning mice remain to be explored, the mechanism is still unclear. This study was designed to investigate the protective effects and mechanism of EORC on lipopolysaccharide (LPS)-induced endotoxin poisoning mice. Pre-treatment with EORC decreased the production of pro-inflammatory cytokines (Interleukin-1β, Interleukin-18, Interleukin-5, and Interferon-γ) and chemokines (Monocyte chemotactic protein-1, Macrophage colony-stimulating factor, and Macrophage inflammatory protein-1β) in serum of endotoxin poisoning mice. The histopathological study showed that the lung injury was improved and EORC decreased the numbers of neutrophils and Nitric oxide (NO) levels in lung. EORC could reduce the mRNA expression of NLR family, pyrin domain-containing 3 (NLRP3), Interleukin (IL)-1β, and nitric oxide synthase (iNOS). In addition, EORC decreased the protein expression of NLRP3, Caspase-1 (p20), Pro-IL-1β, and purinergic P2 × 7 receptor (P2 × 7R) in the lung tissues. The results above indicated that the EORC may have protective effects on LPS-induced endotoxin poisoning mice via inhibiting the activation of P2 × 7R/NLRP3 inflammasome.

1. Introduction

Rimulus cinnamon (Guizhi), the outer bark of *Cinnamonum cassia* Presl(Rougui) has been commonly used to treat phlegm, amenorrhea, cold, and heart palpitations in Chinese herbal clinic. Recent researches show that the anti-inflammatory [1], anti-tumor [2] and anti-viral [3] effects of *Rimulus cinnamon* is assigned part of its activity to essential oils from *Rimulus cinnamon (EORC)*. In addition, the EORC could inhibit the expression of toll-like receptor 2 (TLR2) and the protein tyrosine kinase (PTK) and prostaglandin E_2 (PGE_2) activity [4,5].

Inflammation plays an important role in sepsis [6]. Anti-inflammatory therapy might be effective in the initial phase of sepsis with uncontrolled infection and a hyper-inflammatory response [7]. Lipopolysaccharide (LPS) is a major component of Gram-negative bacteria cell wall, and LPS concentration in blood of patients with sepsis is closely related to the condition and prognosis [8]. After LPS challenge, toll-like receptors (TLRs) recognize the LPS, which lead to the maturation and release of pro-inflammatory response [9]. Overwhelming systemic inflammation causes severe tissue damage, multiple organ failure and even death [10]. Researchers have found that EORC exerts anti-inflammatory effects by down-regulating nuclear factor-kappa B (NF-κB) pathway activation, which reduce the generation and release of inflammatory cytokines [1], Moreover,Toll-like receptors (TLRs) and Nod-like receptors (NLRs) are two major forms of innate immune sensors and closely related to the inflammation [11]. NLR family, pyrin domain containing 3 (NLRP3) is a typical Nod-like receptor. To the best of our knowledge, the protective effects of EORC on LPS-induced endotoxin poisoning mice have not been reported. So, we designed this study to investigate whether EORC protected LPS-induced endotoxin poisoning mice via inhibiting the activation of P2 × 7R/NLRP3 inflammasome.

In this study, endotoxin poisoning mice were induced by LPS. The pro-inflammatory cytokines and inducible chemokines were measured, including Interleukin (IL)-18, IL-1β, IL-5, Interferon-γ (IFN-γ), Monocyte chemotactic protein-1 (MCP-1), Macrophage colony-stimulating factor (M-CSF), Macrophage inflammatory protein-1α (MIP-1α), and Macrophage inflammatory protein-1β (MIP-1β). Lung histopathological study was carried out to study the protective effect of EORC. Moreover, the gene and protein related to NLRP3 inflammasome pathway was investigated.

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Table 1
The constituents in the *Rimulus cinnamon* essential oils.

No.	Retention time (min)	Compound name	The percentage (%)
1	6.004	Benzaldehyde	0.35%
2	7.428	2-Hydroxybenzaldehyde	0.05%
3	7.804	Acetophenone	0.06%
4	8.563	Phenylethyl Alcohol	0.07%
5	9.363	Benzenepropanal	0.41%
6	9.445	endo-Borneol	0.28%
7	9.851	(-)-alpha-Terpineol	0.08%
8	10.333	Cinnamaldehyde, (E)-	1.55%
9	10.498	3-Phenylpropanol	0.12%
10	10.733	2-Methoxybenzaldehyde	0.16%
11	11.445	Cinnamaldehyde	75.26%
12	11.875	Cinnamyl alcohol	0.04%
13	14.527	Coumarin	0.25%
14	14.851	2-Methoxycinnamaldehyde	0.11%
15	15.327	.gamma.-Muurolene	0.04%
16	15.392	Curcumene	0.08%
17	15.721	.alpha.-Muurolene	0.06%
18	15.815	.beta.-Bisabolene	0.08%
19	15.951	(-)-g-Cadinene	0.05%
20	16.074	d-Cadinene	0.11%
21	16.145	2'-Methoxycinnamaldehyde	4.59%
22	16.798	Espatulenol	0.25%
23	16.886	Caryophyllene oxide	0.15%
24	17.433	Espatulenol	0.06%
25	17.592	.alpha.-Cadinol	0.09%
26	17.839	.alpha.-Bisabolol	0.16%
27	18.498	Benzyl Benzoate	0.05%

2. Material and methods

2.1. Preparation and extraction of EORC

The dry *Rimulus cinnamon* samples (about 5000.0 g, dried in shade) were collected from Rong County, Guangxi province, China in November 2015, which were identified by Dr. X.P. Wang, Department of Pharmacognosy in Guiyang College of Traditional Chinese Medicine, China. All voucher specimens were deposited in the Department of Pharmacognosy, Chengdu university of Traditional Chinese Medicine, China. The *Rimulus cinnamon* samples were cut into small pieces, and then the dry samples (each about 100 g) were suspended in 800mL of water to collect the essential oils by steam distillation for 3 h(h). EORC were collected and dehydrated by adding adequate anhydrous sodium sulphate and then centrifuged for 5 min.

2.2. Gas chromatography-mass spectrometry

Chromatographic separation was performed on a gas chromatograph (Agilent technologies 7890 A, Palo Alto, CA, U.S.A.) by using a capillary column Agilent 19091S-433 HP-5 ms 5% Phenyl Methyl Siloxane (30 m × 250 μm × 0.25 μm film thickness) (Agilent, CA, USA). The gas chromatography oven temperature was programmed as follows: kept at 50 °C for 2 min, then adjusted to 120 °C at a rate of 10 °C/min, increased to 150 °C at 5 °C/min, and then ramped to 280 °C at 20 °C/min. The injector temperature was 280 °C. High-purity of helium (99.999%) was used as the carrier gas at a flow rate of 1.0 mL/min, and split injection. ion source temperatures were all set at 230 °C. and the mass scanning range was set from 35 to 550 amu in full scan. The injection was performed by split mode with a split ratio of 40:1. Solvent delay time was set for 3 min for all samples. Compounds were identified by comparison of the obtained mass spectra of the analytes with those of authentic standards from the NIST 2010 library.

2.3. Reagents

Dexamethasone (DEX) and LPS (*Escherichia coil* 055:B5) were provided by Sigma (St. Louis, MO, USA). The MILLIPLEX®MAP Mouse Cytokine/Chemokine Magnetic Bead Panel was available from Merck Millipore (USA). All other reagents were of analytically pure grade.

2.4. Animals

Male C57BL/6J mice (6 weeks old, 18–22 g) were obtained from Beijing Weitong Lihua Biotechnology Co., Ltd. (scxk 2012-0001, Beijing, China) and given free access to water and standard diet in ventilated cages. Mice were housed in a controlled room at 24 ± 2 ℃ and 55 ± 10% humidity under 12 h light–12 h dark cycles.The animals were acclimated for a week before the experiment. The mouse experiment was under the guidelines of the Committee for Animal Care and

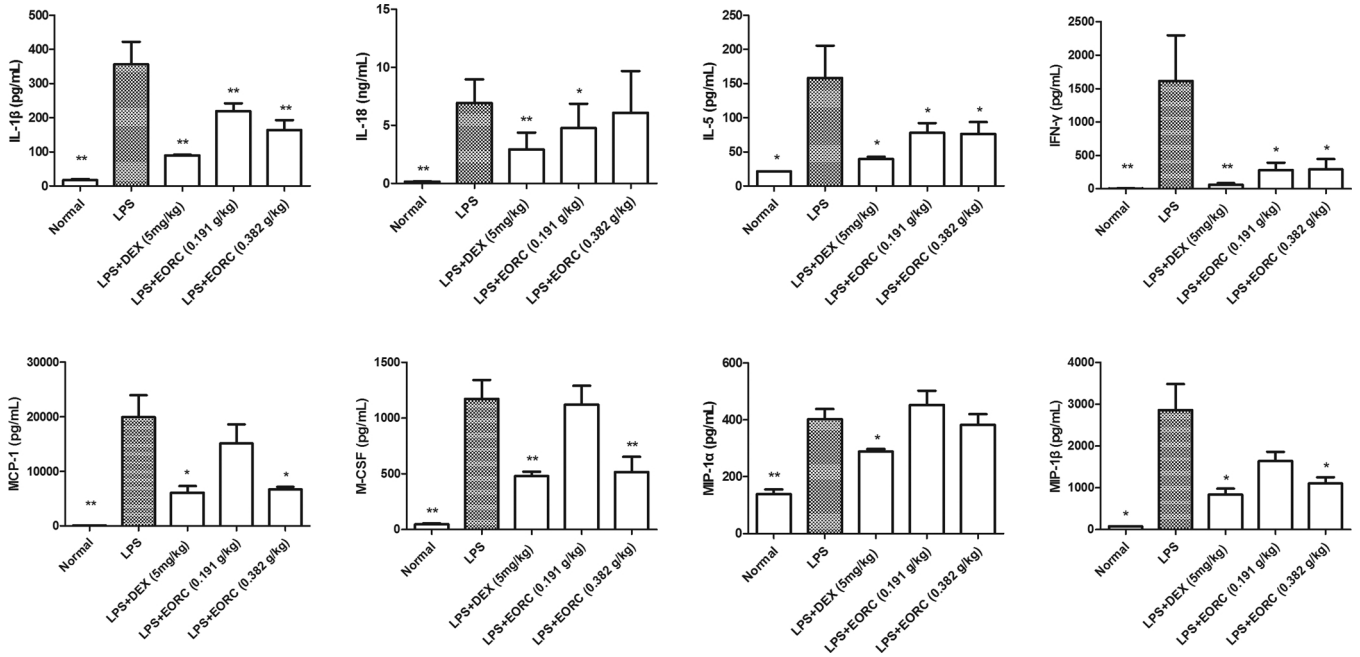

Fig. 1. Effects of EORC on IL-1β, IL-18, IL-5, IFN-γ, MCP-1, M-CSF, MIP-1α, and MIP-1β level in serum. Compared with the LPS group, * $p < 0.05$, ** $p < 0.01$, (IL-18 were measured in each group of 8 ~ 10 animals, and the other cytokines were measured in 4 animals per group).