International Immunopharmacology 61 (2018) 156–161

Contents lists available at ScienceDirect

International Immunopharmacology

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

Leonurine ameliorates the inflammatory responses in lipopolysaccharide-induced endometritis

Haichong Wu[a,b], Ailing Dai[b,c], Xingxing Chen[b,c], Xiaoyan Yang[c], Xiaohua Li[b,c], Cuiqin Huang[b,c], Kangfeng Jiang[a], Ganzhen Deng[a,*]

[a] Department of Clinical Veterinary Medicine, College of Veterinary Medicine, Huazhong Agricultural University, Wuhan, People's Republic of China
[b] Fujian Provincial Key Laboratory for the Prevention and Control of Animal Infectious Diseases and Biotechnology, Longyan 364012, Fujian, China
[c] College of Life Sciences of Longyan University, Longyan 364012, Fujian, China

ARTICLE INFO

Keywords:
Leonurine
Anti-inflammation
Endometritis
Toll-like receptor 4
Nuclear factor-κB

ABSTRACT

Endometritis is the inflammation of the endometrium that is associated with lower conception rates, increased intervals from calving to first service, and more culls for failure to conceive, which leads to serious economic losses in the dairy industry. Leonurine, a natural active compound of *Leonurus cardiaca*, has been proved to possess various biological activities. However, there is still no study about its anti-inflammatory effects on LPS-induced endometritis. The present study aimed to demonstrate the underlying mechanism responsible for the anti-inflammatory effects of leonurine on LPS induced endometritis in mice and in bovine endometrial epithelial cells (bEECs). The results of pathological section displayed that leonurine alleviated LPS induced uterine injury. qRT-PCR and ELISA experiments suggested that leonurine inhibited the expression levels of TNF-α and IL-1β in uterus tissues and bEECs. Molecular studies showed that TLR4 expression and nuclear factor (NF)-κB activation were both inhibited by leonurine treatment. These results suggested that the therapeutic effects of leonurine on LPS-induced endometritis in mice and bEECs may act by inhibiting the expression of TLR4 and its downstream mediated NF-κB pathway. Accordingly, leonurine may serve as an effective drug in preventing and treating LPS induced endometritis.

1. Introduction

Endometritis is the inflammation of the endometrium that is associated with lower conception rates, increased intervals from calving to first service, and more culls for failure to conceive, which leads to serious economic losses in the dairy industry [1–3]. Inflammation of the uterus in cows causes changes in the endometrium, and the Gram-negative bacteria are often considered to be the pathogens that damage the endometrium [4, 5]. Lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria cell wall, has been broadly used in the study of inflammatory models, such as acute lung injury, mastitis, endometritis [6–8]. The pro-inflammatory cytokines secretion, such as IL-1β and TNF-α, will increase sharply in the process of LPS stimulation [9]. It has been reported that NF-κB pathway also activates the transcription of the pro-inflammatory cytokines [10].

Toll-like receptor 4 (TLR4), as an important receptor for LPS, may trigger the activation of an extracellular signaling pathway and contribute to the excessive release of inflammatory cytokines [11]. Numerous studies have demonstrated that TLR4 interacts with molecules, for instance LPS, which are collectively called pathogen associated molecular patterns, and then activate the NF-κB pathway [12, 13].

At present, the major drugs for the treatment of endometritis involve antibiotics, disinfectants, preservatives and hormones [8]. Bacterial resistance, such as *E. coli* resistant to three or more classes of therapeutic antibiotics, and the drug residues are common consequences of the long-term use of these drugs [1, 14]. Thus, it is necessary to explore new methods for the treatment of endometritis in dairy cows.

Currently, due to the fewer side effects of natural products, it has been favored by many researchers and used to treat many diseases such as cancer, diabetes, inflammation [6, 15, 16].

Leonurine (the chemical structure is shown in Fig. 1A), a natural active compound of *Leonurus cardiaca*, has been proved to possess various biological activities, including cardioprotective, anti-oxidative, anti-apoptotic, as well as anti-inflammatory [17–20]. However, there is still no study about its anti-inflammatory effects on LPS-induced endometritis.

In this study, we mainly investigated the anti-inflammatory effects

* Corresponding author.
E-mail address: ganzhendeng@sohu.com (G. Deng).

https://doi.org/10.1016/j.intimp.2018.06.002
Received 26 March 2018; Received in revised form 9 May 2018; Accepted 1 June 2018

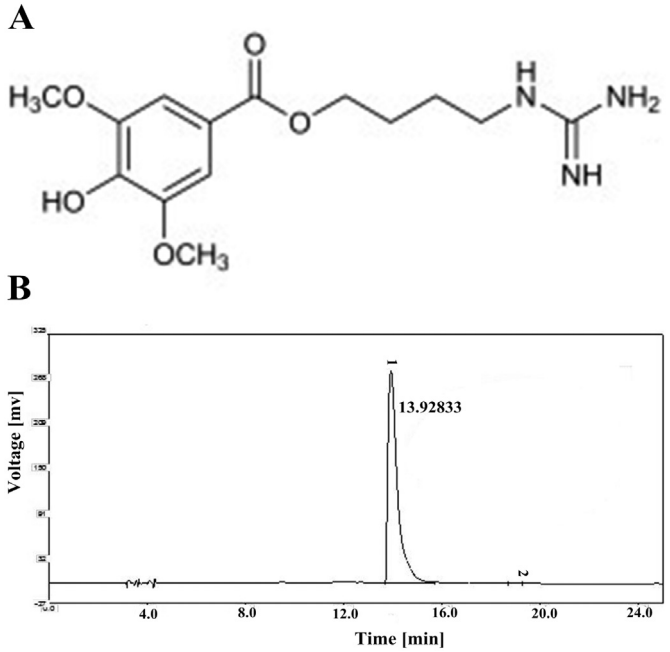

Fig. 1. Chemical structure of leonurine.

Table 1
Primers used for quantitative real-time PCR.

Name	Sequence (5′ → 3′): forward and reverse	GenBank accession no.	Product size (bp)
TNF-α	CTTCTCATTCCTGCTTGTG ACTTGGTGGTTTGCTACG	NM_013693.3	198
IL-1β	CCTGGGCTGTCCTGATGAGAG TCCACGGGAAAGACACAGGTA	NM_008361.4	131
GAPDH	CAATGTGTCCGTCGTGGATCT GTCCTCAGTGTAGCCCAAGATG	NM_ 001289726.1	124

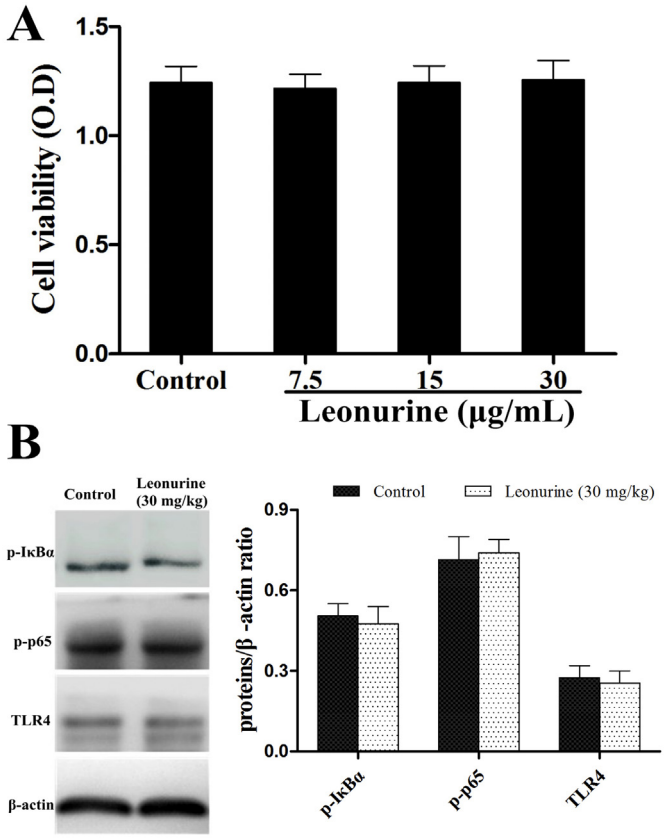

Fig. 3. Effects of leonurine on cell viability and uterus tissues. A. Effects of leonurine on cell viability. Bovine endometrial epithelial cells were cultured with various concentrations of leonurine (0–30 μg/mL) for 24 h. Cell viability was determined by MTT method. B. Effects of leonurine (30 mg/kg) alone on uterus tissues. Data are represented as the mean ± S.E.M. of three independent experiments. $^{\#}p < 0.05$ *vs.* Control group, $^{*}p < 0.05$ *vs.* LPS group.

of leonurine on pro-inflammatory cytokines secretion in LPS-induced endometritis in mice and bovine endometrial epithelial cells (bEECs). Moreover, the molecular mechanisms involved were also explored.

2. Materials and methods

2.1. Reagents

Leonurine (purity ≥ 98%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The purity of leonurine was determined with high performance liquid chromatography (HPLC). The study was performed on EChrom2000 DAD Data System. Chromatography was performed through a Sinochrom 0DS2 C18 column (4.6 × 250 mm, 5 μm). Elution was performed with 0.001 M ammonium acetate/acetonitrile, and the flow rate was 1.0 mL/min with DAD detection at 275 nm (Fig. 1B). LPS was purchased from Sigma (USA). IL-1β and TNF-α ELISA kits were obtained from BioLegend (USA). All antibodies used in this study were purchased from Cell

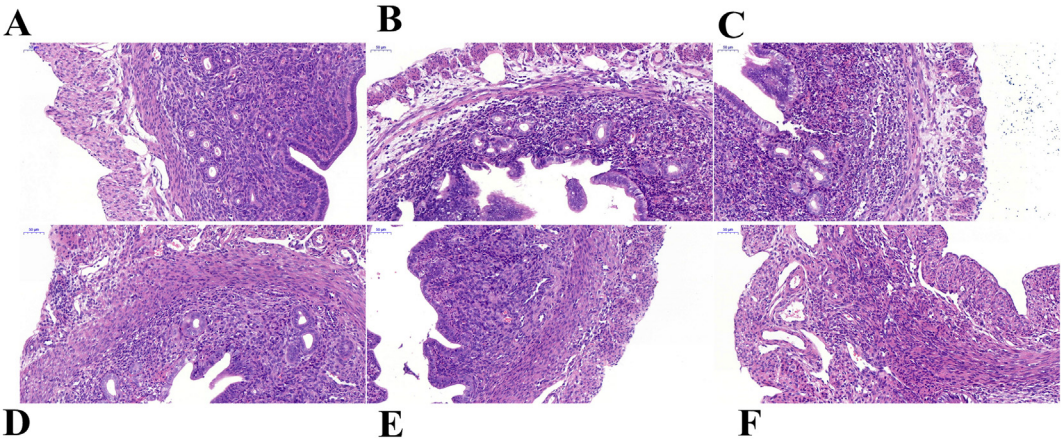

Fig. 2. Effects of leonurine on LPS-induced uterine injury. (A). Control group. (B). LPS group. (C–E). LPS + leonurine (7.5, 15, 30 mg/kg) groups. (F). Dexamethasone + LPS group (Dex group).