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GAVIN PUBLISHERS

International Journal of Applied Research on Medicinal Plants

Oyuntsetseg N, et al. Int J Appl Res Med Plants: IJARMP-106.

DOI :10.29011/ IJARMP -106. 100006

Research Article

Effect of De-Mon Syrup on Lipopolysaccharide-Induced Acute Lung Injury in Rats

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Citation: Oyuntsetseg N, Chimedragchaa C, Nomin-Erdene J, Tsend-Ayush D (2018) Effect of De-Mon Syrup on Lipopolysaccharide-Induced Acute Lung Injury in Rats. Int J Appl Res Med Plants: IJARMP-106. DOI :10.29011/ IJARMP -106. 100006

Received Date: 04 December, 2018; **Accepted Date:** 10 December, 2018; **Published Date:** 21 January, 2019

Abstract

In traditional medicine, acute lung injury is considered as a hot-natured condition caused by impure blood, bile and microbes. Deva-5 is one of the multi-component compounds used for treatment of hot-natured conditions in traditional medicine, and is composed of 5 ingredients including *Gentiana decumbens* L., *Momordica cochinchinensis* L., *Chiazospermum erectum* Bernh., *Polygonum bistorta* L. and *Terminalia chebula* Retz. In our prior study, Deva-5 has been shown to have antiviral, antibacterial and anti-inflammatory properties. A syrup medicine, De-Mon, is produced containing the ingredients of Deva-5. The present study examines the effects of De-Mon on LPS-induced acute inflammation of the lungs. Acute inflammation of the lungs was induced in rats by intravenous injection of Lipopolysaccharide (LPS) at the dosage of 7.5 mg/kg. De-Mon was given orally to rats at the dosage of 39.6 mg/kg for 5 days prior to the LPS administration. Blood plasma levels of cytokines such as TNF-α, IL-1β, IL-6 and IL-10 were determined by enzyme-linked immunosorbent assay. Lung tissues were obtained 24 hours after LPS administration and subjected to histopathological analysis. De-Mon significantly decreased LPS-induced increases of serum levels of TNF-α, IL-1β and IL-6 compared to control ($p<0.01$). Whereas, serum levels of IL-10 were notably increased due to De-Mon treatment ($p<0.01$) in rats administered LPS. Hyperemia of alveolar capillaries, inflammatory exudates in the alveoli and mononuclear cell infiltration in the bronchioles and alveoli were reduced by the effect of this treatment. De-Mon was proved to reduce lung inflammation by inhibiting TNF-α, IL-1β and IL-6 and increasing activity of IL-10 in rats administered LPS.

Keywords: Acute Lung Injury; Cytokines; De-Mon; Hot-Natured Disorders; LPS; Traditional Medicine

Introduction

Traditional Mongolian Medicine (TMM) has developed over thousands of years and greatly influenced by nomadic lifestyle and frequent exposure to severe climate conditions [1-3]. In TMM, decoction is most widely used form and preferable way of preparing decoction is boiling of the drugs with water. This helps in selective release of water-soluble active components making it less toxic. Moreover, such aqueous extracts are effective, absorbs quickly and completely with high bioavailability. However, the decoction is trouble to prepare, and inconvenient to carry and store [4]. Because of these different forms such as granules, capsules, tablets and syrups are developed as alternatives to decoctions and used widely by traditional practitioners in many parts of the world including China, Japan, Korea, US and some European countries.

In traditional medicine, acute lung injury is considered as hot-natured condition caused by impure blood, bile and microbes [5,6]. Deva-5 decoction is one of the multi-component compounds used for treatment of hot-natured conditions in traditional Mongolian medicine [5]. It is composed from 5 ingredients including *Gentiana decumbens* L., *Momordica cochinchinensis* L., *Chiazospermum erectum* Bernh., *Polygonum bistorta* L. and *Terminalia chebula*

Retz [5,6]. Main components of Deva-5 have been shown to have antiviral, antibacterial and anti-inflammatory properties [7-15]. From our recent study on anti-viral effect of Deva-5, we found that *Ch.erectum*, *T.chebula* and *M.cochinchinensis* have high anti-viral activity against H3N8 and might be a promising potential source of new anti-viral agents [16].

De-Mon is syrup medicine produced with the ingredients of traditional recipe named Deva-5. Based on our previous study results, we postulated that De-Mon syrup could protect against LPS-mediated Acute Lung Injury (ALI). In the present study, we tested this hypothesis using a rat model of LPS-mediated ALI.

Material and methods

Preparation of De-Mon

The crude herbal medicines from *Gentiana decumbens* L., *Momordica cochinchinensis* L., *Chiazospermum erectum* Bernh., *Polygonum bistorta* L. and *Terminalia chebula* Retz. were purchased from Traditional Drug Factory at the Institute of Traditional Medicine and Technology of Mongolia. All plant materials were size reduced into coarse powder (3 mm) and macerated with 20% ethanol. Then the extract was filtered out and concentrated under vacuum using rotary vacuum evaporator. The residue obtained was then subjected for further extraction processes. Herbal syrup was prepared according to standard formulae and all evaluation techniques have been carried out as per standards.

Reagents

Escherichia coli 055:B_5 endotoxin from Sigma-Aldrich and the cytokine immunoassay kits from Shanghai MLBIO Biotechnology Co. Ltd. (China) were used in the study. All other solvents and chemicals were of analytical grade.

Experimental animals

A total of fifty 8-10-week-old male Wistar rats (180-220 g) were used in this study. All experimental animals were obtained from the Experiment Animal House, Institute of Traditional Medicine and Technology. The rats were housed in cages and maintained at room temperature with a 12-h light/dark cycle. They were fed with standard pellet diet and tap water ad libitum.

Experimental protocols

Rats were randomized into three groups: control group (n=20), LPS group (n=20), in which LPS (7.5 mg/kg dissolved in 0.5 mL sterile saline) was administered by an intravenous injection (iv) via the tail vein; and LPS+ De-Mon syrup group (n=20), in which De-Mon syrup (39.6 mg/kg, orally) was given for 5 days before injection of LPS (7.5 mg/kg dissolved in 0.5 mL sterile saline, iv) orally [17]. Rats were euthanized with an overdose of ketamine hydrochloride (80 mg/kg, ip). Lung tissue specimens and blood samples were then obtained for further analysis.

Plasma levels of cytokines (TNF-a and IL-1β, IL-6, IL-10)

Blood samples were collected via cardiac puncture at 3, 6, 9 and 12 h after the administration of LPS and from healthy rats. All rats were euthanized with ketamine hydrochloride before blood collection. The collected blood samples were centrifuged at 377.3 g for 10 min at 4˚C, and the plasma supernatant was stored at -20˚C until further analysis. The plasma levels of TNF-a and IL-1β, IL-6, IL-10, were detected using solid-phase sandwich enzyme-linked immune sorbent assay (ELISA, Shanghai MLBIO Biotechnology Co. Ltd.) kits specific for the detection of these factors, and the absorbance was measured at 450 nm by a plate reader (Chromate 4300 microplate, Shanghai MLBIO Biotechnology Co. Ltd., China).

Histological analysis

Twenty-four hours after LPS administration, the rats were euthanized (n=5, 3, and 5 in the control, LPS, and LPS +De-Mon groups, respectively). The obtained lung tissue specimens were fixed with 10% formalin, embedded in paraffin, cut into 5-mm thick sections and mounted onto slides. The sections were then stained with hematoxylin and eosin (H&E) according to the standard staining method [18]. Histologic changes were graded by a pathologist blind to the clinical status of the rats. Then the lung tissue samples were scored for the degree of intra-alveolar edema, intra-alveolar hemorrhage, and neutrophil infiltration using grades 0 to 4 (0, absent; 1, mild; 2, moderate; 3, severe; 4, overwhelming) with a maximum score of 12, as described previously [19].

Statistical analysis

Data are reported as mean±SD. Statistical significance was determined by one-way analysis of variance followed by Tukey's multiple comparison test. A P value <0.05 was considered statistically significant.

Results

Effects of De-Mon on serum level of TNF-α, IL-1β, IL-6 and IL-10

The *in vivo* anti-inflammatory activity of De-Mon was monitored by evaluating the levels of inflammatory cytokines. The levels of TNF-α, IL-1β and IL-6 level increased significantly in plasma after LPS administration compared with the control group and peaked at 3 h and 9 h respectively. Pre-treatment with De-Mon significantly suppressed the production of TNF-α (LPS + De-Mon group vs LPS group: $p<0.01$ at 3 h and 9 h), IL-6 (LPS + De-Mon group vs LPS group: $p<0.05$ at 9 h) and IL-1β (LPS + De-Mon group vs LPS group: $p<0.01$ at 3 h, 6 h, 9 h and 12 h) at the indicated time points (Figures 1A-C). Meanwhile, treatment with De-Mon induced the LPS-dependent IL-10 anti-inflammatory

cytokine. The levels of IL-10 increased gradually and reached a peak at 12 h and was significantly different compared with the LPS group (LPS + De-Mon group vs LPS group: $p<0.01$ at 3 h, 6 h, 9 h and 12 h) (Figure 1D).

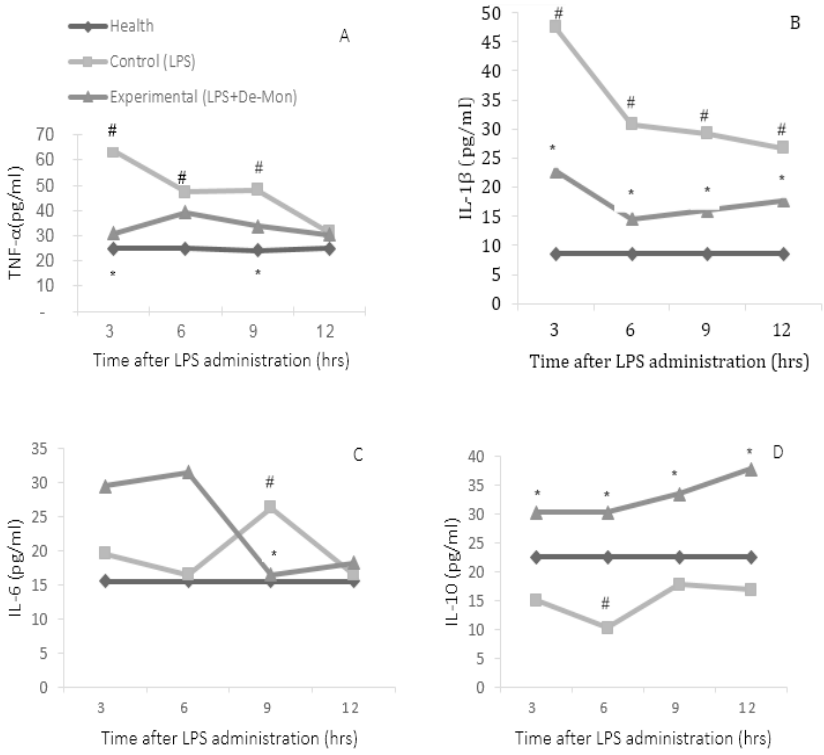

Figure 1: Effects of De-Mon on the production of plasma levels of TNF-α, IL-6, IL-1β and IL-10 after LPS administration. Rats were given De-Mon orally during 5 days prior to an i.v. administration of LPS. Blood was collected at 3 h, 6 h, 9 h and 12 h following LPS challenge to analyze the cytokines of TNF-α (**A**), IL-6 (**B**), IL-1β (**C**) and IL-10 (**D**). The values presented are mean ± SD (n=20 in each group). #$p<0.01$, control (LPS) group compared to health group; * $p<0.05$ De-Mon + LPS group vs. LPS group.

Pre-treatment with De-Mon attenuated LPS-mediated lung histopathological changes

To evaluate the histological changes, lung tissues harvested at 24 h after LPS administration, were stained with H&E. As shown in Figure 2A,B, lung tissues from the control group showed normal structure and no histological alterations. In the LPS-group, the lung tissues had significant histo pathological changes, such as thikening of the alveolar wall, bleeding and inflammatory cell infiltration (Figures 2C,D). Rats pre-treated with De-Mon showed less inflammation and distortion of lung architecture indicating that acute lung injury was attenuated by treatment (Figures 2E, F).

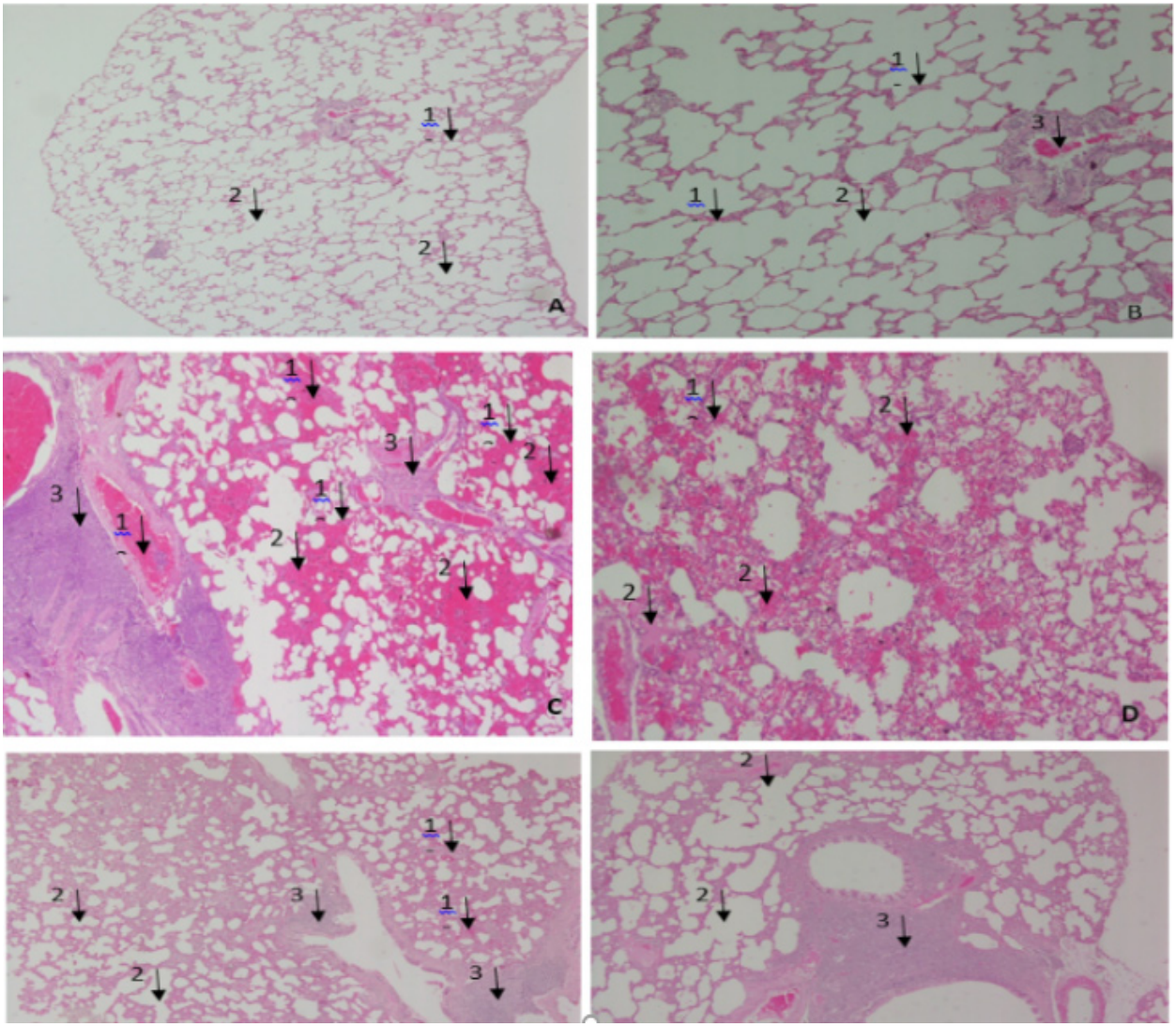

Figure 2: Histological feature of lung tissue after 24 hours since LPS administration

A, B, Histological feature of lung in health group. C, D, Histological feature of lung in control (LPS) group. E, F, Histological feature of lung in experimental (LPS+De-Mon) group.1, Alveolar wall.2, Alveoli.3, Bronchiole. Staining: Hematoxylin and eosin, x40, x100.

Discussion

In traditional Mongolian medicine, acute lung injury is considered as hot-natured condition caused by impure blood, bile and microbes [5]. Deva-5 is one of the multi-component compounds used for treatment of hot-natured conditions in traditional medicine [5-6]. In current study, the protective effect of De-Mon, syrup version of traditional medicine Deva-5 on LPS-induced Acute Lung Injury (ALI) or Acute Respiratory Distress Syndrome (ARDS) was evaluated. Our data demonstrated that pre-treatment with De-Mon decreased pro-inflammatory cytokines production and attenuated lung tissues damage. This suggests that De-Mon might be potential therapeutic candidate for the ALI.

LPS-mediated ALI is characterized by pro-inflammatory cytokines activation which play important role in development and progression of ALI. IL-1β, for example, inhibits fluid transportation across the distal lung epithelium to cause surfactant abnormalities and increases protein permeability across the alveolar-capillary membrane. The levels of TNF-α, IL-1β and IL-6 increased in patients with ALI or ARDS [20,21]. These cytokines initiate and amplify the inflammatory response in ALI. In this study, we found that De-Mon significantly attenuated the production of TNF-α, IL-1β, and IL-6 and induced IL-10

production in LPS-mediated Acute Lung Injury. Therefore, our results suggested that the protective effects of De-Mon on LPS-mediated Acute Lung Injury may be attributable to the inhibition of inflammatory cytokines and induction of IL-10.

Anti-inflammatory properties of De-Mon components have been studied at some degree by others. Triterpenoidal saponin isolated from the seeds of *Momordica cochinchinensis* inhibits LPS-induced expression of IL-6 in macrophages. Quillaic acid glycoside isolated from the seeds of *Momordica cochinchinensis* inhibit LPS-induced expression of NO and IL-6 in macrophages nuclear factor-κB pathway. Protopine isolated from *Chiazospermum erectum* attenuates LPS-induced inflammatory responses in murine macrophages, and reduced the production of nitric oxide, inducible nitric oxide synthase, cyclooxygenase-2, and pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6 via the inhibition of phosphorylation of extracellular-signal-regulated kinases 1/2 and *c-Jun* N-terminal kinase and inhibition of nuclear factor-κB activation in LPS-activated macrophages [22]. From our previous study on anti-viral effect of Deva-5, we found that *Ch.erectum*, *T.chebula* and *M.cochinchinensis* which are main components of De-Mon have high anti-viral activity against H_3N_8 and might be a promising potential source of new anti-viral agents [16]. *Ch.erectum*, *T.chebula* and *M.cochinchinensis* are rich in alkaloids and flavonoids, thus, we speculate that these components had a major contribution to the effects observed in this study.

In conclusion, De-Mon syrup, developed from the traditional Mongolian recipe reduces lung inflammation by inhibiting TNF-α, IL-1β and IL-6 and increasing activity of IL-10 in rats administered LPS. Our results suggest that De-Mon could be a potential medicine for preventing and treating LPS induced ALI.

Competing interests

The authors declare that they have no competing financial interests.

Acknowledgements

This study was supported by the Mongolian Foundation for Science end Technology (MFST; agreement: IN_A/178-10/2016).

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