Bojungikgi-tang Alleviates Lipopolysaccharide-Induced Inflammatory Responses in RAW264.7 Macrophages and C57BL/6 Mice

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Background: This study evaluated the anti-inflammatory effect of Bojungikgi-tang (BJT) in a model of acute lung injury (ALI) in mice.

Methods: Murine cell line macrophages (RAW264.7) were treated with BJT for 30 minutes before lipopolysaccharide (LPS) treatment. The levels of cytokines, mRNAs, proteins, and related markers were investigated. In addition, BJT (200 mg/kg/day) was administered orally to C57BL/6 mice for 2 weeks prior to an intraperitoneal injection with LPS to induce sepsis and ALI; 24 hours post-LPS injection, mice were sacrificed and blood was collected from the infraorbital vein. Lung tissue was harvested, hematoxylin and eosin staining was performed, the wet/dry ratio of the lung tissue was measured, and the serum cytokine levels were analyzed.

Results: Compared with LPS treatment, BJT suppressed LPS-induced mRNA expression and secretion of inflammatory cytokines in RAW264.7 macrophages. Furthermore, inducible nitric oxide synthase, cyclooxygenase-2, toll-like receptor 4, phosphorylation of mitogen-activated protein kinases, and phosphorylation of nuclear factor kappa-light-chain-enhancer of activated B cells were inhibited by BJT. In mice, LPS-induced pathological changes in lung tissues, such as abnormal histological structures, immune cell infiltration, and lung edema were less severe following BJT treatment. BJT inhibited the LPS-induced increase of cytokines such as interleukin 4, 6, 10, and tumor necrosis factor alpha.

Conclusion: BJT had an inhibitory effect in the pathological progress of LPS-induced sepsis and ALI and may be a promising therapeutic agent in the future.

Keywords: acute lung injury, lipopolysaccharide, macrophage, sepsis

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Introduction

Sepsis is a profound, systemic inflammatory response to infection, is caused by bacterial, viral, fungal, or parasitic pathogens, and the condition is characterized by a fever/hypothermia, tachycardia, tachypnea, pathologic leukocytosis, or leukopenia. Sepsis continues to pose a global health challenge. It causes 6 million deaths annually. Considering the severity of its impact, more emphasis needs to be placed on research and management of sepsis. Sepsis-related mortality has continued to increase in South Korea over the last 10 years, resulting in a sharp increase in associated social burdens [1].

The symptoms of sepsis vary depending on the type of pathogen that causes the infection, but the leading cause of sepsis-related death is multiple organ damage by systemic inflammatory response syndrome [2]. These vital organs include the lungs, liver, and kidneys, and in the event of acute lung injury (ALI), the condition may lead to acute respiratory distress syndrome, adversely affecting the survival of patients [3].

There are animal models for testing treatments for sepsis and these include bacterial injection (intraperitoneal or intravenous) of mice to mimic sepsis, surgical trauma to cecal or colon tissue, and injection of the bacterial endotoxin lipopolysaccharide (LPS) [4].

Sepsis may be caused by various types of pathogens, therefore in Korean traditional medicine, it is difficult to identify how to characterize sepsis. Sepsis was categorized as a sinking pattern, a pathological symptom in which toxic factors enter from the skin into the body and is an externally acquired condition/disease. It is caused by an abundance in Xie Qi (pathogenic energy) or deficiency in Zheng Qi (vital energy), and has a poor prognosis. When qi...
from the spleen is weak, caused by damage to the spleen (which governs the raising of the middle energizer), the result is a loss of power to raise energy and achalasia of the affected tissue and visceral organ, and prolapse can develop.

In terms of collapse of middle warmer energy, symptoms of sepsis that accompanies primary symptoms are shortness of breath and lack strength, bloated tummy after eating, abdominal heaviness, constant urgency of defecation, rectum prolapse due to prolonged diarrhea, astropesis, and uterine prolapse, and secondary symptoms such as dizziness, low voice, spontaneous perspiration, reduced appetite, tired of mind, runny feces, pale tongue with thin and white coating, and weak pulse [5].

Bojungikgi-tang (BJT) is an herbal prescription with the following medicinal plants in its composition: Astragali radix (Huang Qi), Glycyrrhizae radix (Gan Cao), Ginseng radix (Ginseng, Ren Shen), Atractylodes Rhizome White (Bai Zhu), Angelicae radix (Dang Gui), Citrus Unshiu Peel (Chen Pi), Cimicifugae rhizome (Sheng Ma), and Bupleuri radix (Chai Hu) [5].

This study examined the effect of BJT on LPS-induced inflammatory responses in vitro in a murine macrophage cell line. LPS-induced sepsis in mice was also studied to examine if there was a protective effect of BJT against ALI due to pretreatment. Pathological damage in lung tissue, among the pathological findings of ALI, and the regulatory effect on the production of inflammatory cytokines was also determined.

Materials and Methods

1. Preparation of sample solution

The BJT (Hanpoong Bojungikgitang soft extract, Hanpoong Pharmaceutical Co. Ltd., Jeonju-si, Republic of Korea) was dissolved in phosphate buffered saline (PBS). The concentration of BJT (23.5% of the total weight) excluded purified water and excipients such as liquid fructose and fructooligosaccharides.

2. Cell culture of RAW264.7 macrophages and cytotoxicity assay

RAW264.7 (American Type Cell Collection, Manassas, VA, USA), a murine macrophage cell line, was cultured in Dulbecco’s Modified Eagle’s Medium containing 10% fetal bovine serum, penicillin and streptomycin, and maintained at 37°C in an incubator with 5% CO2, and a relative humidity of 95%.

LPS was dissolved in PBS, and the RAW264.7 cell line was treated with LPS1 μg/mL, and BJT (23.5% of the total weight) treatment which was performed 30 minutes before the LPS treatment.

Cell proliferation was determined using the cell viability, proliferation, and cytotoxicity assay (MTT assay). RAW264.7 macrophages were seeded at 5 x 10^4/mL in a 96-well plate. After 24 hours, the cells were treated with BJT at various concentrations (0, 50, 100, 200, 500, 1,000 μg/mL) for 30 minutes and with LPS for 24 hours, after which, the cells were treated with MTT solution for 1 hour to dissolve the formazan, which is directly proportional to the number of viable cells, and the absorbance was measured at a wavelength of 570 nm using a spectrophotometer (Molecular Devices).

3. Animals

Six-week-old C57BL/6 mice (Dae Han Bio Link, Eumseong Chungbuk, Korea) with a mean body weight of 22 g were acclimated for 7 days under constant temperature and humidity. Then, BJT was orally administered for a total of 2 weeks at a dose of 200 mg/kg/day. 15 mg/kg LPS (Sigma-Aldrich) was injected intraperitoneally to induce sepsis in the mice, and 24 hours post induction of sepsis, blood was collected from the infraorbital venous plexus whilst the mice were under carbon dioxide asphyxiation. After which, the mice were sacrificed through cervical dislocation.

All animal care protocols and experimental procedures were approved by the Animal Care and Use Committee of Kyung Hee University (Approval no.: KHSASP-22-131) in compliance with the Principle of Laboratory Animal Care of the National Institutes of Health.

4. Hematoxylin and eosin staining of the lung tissue

Before performing hematoxylin and eosin staining, the slide was deparaffinization by being dipped twice, for 5 minutes, in a jar containing xylene. To hydrate the tissue section, it was dipped into decreasing concentrations of ethanol baths (100%, 90%, 80%, and 70%) for 5 minutes, respectively, and left in distilled water (D.W.) for 1 minute.

To stain with hematoxylin the slide was saturated for 2 minutes and 30 seconds, and washed in running D.W. multiple times, followed by being dipped in 0.3% HCl-alcohol 1-2 times, and rinsed in running D.W before it was dipped in 0.1% ammonia water for 30 seconds and rinsed in running D.W. The slide was then stained in eosin in 0.1% acetic acid for 2 minutes, washed in increasing concentrations of ethanol (70%, 95%) for 1 minute, respectively, and put in a xylene bath for 1 minute for clearing, and repeated. The cover-slide was attached to observe using light microscopy (×200) under the EVOS microscope (Thermo Fisher).

5. Wet-to-dry lung weight ratio

The lung wet-to-dry weight ratio was used as an index of
lung edema from the lung injury induced by injection of LPS into the mice. The weight of the extracted lung tissue was measured immediately and referred to as the wet weight. The lung tissue was dried in an oven at 60ºC for 48 hours, and the weight was measured and referred to as the dry weight. The wet/dry ratio was calculated for each mouse lung.

6. RT-PCR

Total ribonucleic acid (RNA) was extracted using R&A Blue Total RNA Extraction kit (iNtRON Biotech, Korea), and Nanodrop 1,000 spectrophotometer (Thermo Fisher Scientific) was used to determine concentration of RNA. Complementary deoxyribonucleic acid was generated using a synthesis kit (Takara Bio Inc., Kusatsu, Japan) for 1 µg of RNA. The sequence of primers used in this study are listed in Table 1.

7. Western blotting

After culturing of RAW264.7 macrophages, the cells were washed with PBS and lysed in ice cold radioimmunoprecipitation assay buffer [150 mM NaCl, 1% NP-40, 0.5% DOC, 0.1% SDS, 50 mM Tris (pH 8.0), 1 mM, 1 mM , 1 mM, 1 mM, and 1 µg/ml aprotinin, leupeptin, pepstatin)]. Insoluble residues were removed by centrifugation (13,000× g, 20 minutes, 4ºC), and the concentration of the extracted protein was determined using the Bradford protein assay [6]. After mixing 15 µg of protein with 6× sample buffer, and boiling at 95ºC for 5 minutes, electrophoresis was performed on a 10% sodium dodecyl sulfate-polyacrylamide gel, and the separated protein was transferred onto a nitrocellulose membrane. The nitrocellulose membrane was blocked in 2% skimmed milk and 2% bovine serum albumin, and then incubated at 4ºC for 12 hours or longer with primary antibodies diluted at 1:1,000. The membrane was then incubated with the appropriate secondary antibodies (1:4,000) for 1 hour at room temperature. Thereafter, protein bands were visualized using an enhanced chemiluminescence detection kit (DoGEN, Seoul, Republic of Korea).

8. Enzyme linked immunosorbent assay

For analysis of the levels of cytokine expression detected in the cell culture and serum, mouse interleukin (IL)-4, IL-6, and tumor necrosis factor alpha (TNF-α) Quantikine ELISA kits were used (R&D Systems), and IL-8 ELISA kit (MyBioSource) was used.

9. Statistical analysis

For all experimental results, three or more replicates were performed and the results were presented as mean ± SD. Statistical significance of the results was tested and analyzed using one-way ANOVA, and the significance level was \( p \leq 0.05 \).

Results

1. Effect of BJT on cell proliferation inhibition in LPS-treated RAW264.7 macrophages

LPS, a glycolipid constituting the outer membrane of Gram-negative bacteria, is a potent activator of immune responses in immunocytes. LPS triggers secretion of many different inflammatory cytokines from macrophages, contributing to the pathogenesis of sepsis [7]. In this process,
cell proliferation is observed in LPS-treated macrophages [8]. Treatment with BJT prior to treatment with LPS (1 μg/mL) inhibited the growth of RAW264.7 macrophages (Fig. 1). Based on the results of the MTT assay, for further experiments, BJT up to a concentration of 500 μg/mL was used.

2. Effect of BJT on suppressing inflammatory cytokines in LPS-treated RAW264.7 macrophages

Inflammatory cytokines play a major role in the complex pathophysiology underlying sepsis and knowledge on the actions of these cytokines can be useful in diagnosis, prognosis prediction, and therapeutic targeting of sepsis [9].

In LPS-treated (1 μg/mL) RAW264.7 macrophages, a significant increase in the messenger ribonucleic acid (mRNA) levels of inflammatory cytokine-related genes, IL-4, IL-6, IL-8, and TNF-α, was observed. Prior treatment with BJT 200 μg/mL resulted in a reduced expression of these genes (Fig. 2A). In addition to assessing expression of mRNA levels of inflammatory cytokines (IL-4, IL-6, IL-8, and TNF-α) these cytokines were measured in the cell culture using BJT 200 μg/mL and most cases BJT 500 μg/mL treatment prior to LPS (1 μg/mL) treatment. The levels of inflammatory cytokines increased significantly with LPS treatment, but where BJT treatment was given 30 minutes before LPS, inflammatory cytokine levels were lower (Fig. 2B).

3. Effect of BJT on suppressing protein expression related to inflammatory responses in LPS-treated RAW264.7 macrophages

When inflammatory responses are induced in LPS-treated (1 μg/mL) RAW264.7 macrophages, expression of various pro-inflammatory mediators and inflammation-related factors is induced. Thus, through Western blotting, the effect of 200 and 500 μg/mL BJT treatment was examined for these mediators and factors. The results showed that BJT treatment prior to LPS treatment resulted in a lower expression of inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2) and toll-like receptor 4 (TLR4), compared with LPS treatment (Fig. 3A). In addition, BJT treatment prior to LPS treatment had lower levels of...
expression for phosphorylation of extracellular signal-regulated kinase, Jun N-terminal kinase and p38, which are mitogen-activated protein kinases (MAPK), playing key roles in inflammatory responses compared with LPS treatment. Furthermore, BJT treatment prior to LPS treatment showed less expression of phosphorylation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), recognized as the central dogma of inflammatory responses, compared with LPS treatment (Fig. 3B).

4. Effect of BJT on improvement of lung tissue injury in LPS-induced septic mice

Mice injected intraperitoneally with 15 mg/kg of LPS were sacrificed 24-hour post injection, and lung tissue was extracted to observe changes in histopathology. As a result of examining the overall extent of lung injury using hematoxylin and eosin staining, infiltration of many inflammatory cells was observed throughout the alveoli in the LPS-treated group, and bleeding from the pulmonary microvasculature into the alveoli, interstitial thickening and edema were also observed. On the other hand, in the BJT-treated group (200 mg/kg/day for 2 weeks) prior to LPS treatment (intraperitoneally with 15 mg/kg of LPS), edema and immune cell infiltration were lower compared with the LPS group (Fig. 4A).

The wet/dry, ratio was 9.70 ± 0.69 in the LPS-treated group, and 8.40 ± 0.37 in the BJT-treated (200 mg/kg/day for 2 weeks) prior to LPS treatment group, but there was no statistical significance between these groups (Fig. 4B).

5. Effect of BJT on serum cytokine levels in LPS-induced septic mice

In order to examine the effect of BJT on inflammatory responses, serum cytokine levels were measured by separating serum from blood collected from the infraorbital venous plexus of mice. As a result, the expression of serum pro-inflammatory cytokines IL-4, IL-6, IL-8 and TNF-α of mice was significantly higher in LPS-induced (intraperitoneally with 15 mg/kg of LPS) septic mice compared with the control mice group. On the other hand, for the mice group
treated with BJT (200 mg/kg/day for 2 weeks) prior to LPS treatment, these cytokine levels in the serum showed significantly lower levels compared with those in the LPS-induced septic mice group (Figs. 5A-5D).

**Discussion**

Sepsis is a systemic inflammatory response to microbial infection of which there are 3 stages (sepsis, severe sepsis, and septic shock). When bacterial pathogens from one part of the body enter the bloodstream and travel to other parts of the body, sepsis may then develop and the patient has a systemic inflammatory response. Severe sepsis that has progressed to septic shock and is accompanied by multiple organ failure is a life-threatening condition. Globally the estimated associated mortality from sepsis is high and probably underestimated due to lack of availability of reliable statistics from low-income countries [10]. An extrapolation of data based on statistics from high-income countries projects that 31.5 million cases of sepsis occur globally each year (1), with associated mortality in the range of 25-50% [11].

Data collection through the web-based sepsis data platform built by the Korea Sepsis Alliance showed that out of a total of 64,021 patients aged 19 years or older who visited the emergency room of 19 university hospitals nationwide during the period of one month in January 2018, 977 patients (1.5%) had sepsis, of which 357 patients had septic shock [12]. In addition, as a result of analyzing 2,125 cases of sepsis registered for 6 months from September 2019 to February 2020, 1,720 cases (80.9%) were community onset sepsis, and 405 cases (19.1%) were hospital onset sepsis [12]. Moreover, there were 371.8 sepsis cases per 100,000 patients visiting the emergency room, and 18.0 cases per 100,000 inpatients. The sepsis-related mortality rate is 28.6% in South Korea, which is still high compared to that of foreign countries [12].

Sepsis is defined as a potentially life-threatening medical emergency where the patient has two or more of the following: (1) oral body temperature > 38°C or < 36°C; (2) heart rate > 90 beats per minute and respiratory rate > 20 breaths per minute; (3) white blood cell count > 12,000 cells/mm³ or < 4,000/mm³; and (4) band cell count > 10% [13]. These symptoms of sepsis are caused by the infection, and/or their toxins which spread to the cardiovascular system. In response to the infection, sepsis may be further complicated

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**Fig. 5. Effect of BJT on serum cytokine levels in LPS-induced septic mice.** ELISA was performed to determine the serum levels of (A) IL-4; (B) IL-6; (C) IL-8; and (D) TNF-α.

### p < 0.001.

#### p < 0.0001 vs. control group.

**p < 0.01.

##### p < 0.001 vs. LPS group.

BJT = Bojungikgi-tang; LPS = lipopolysaccharide.
by organ damage or dysfunction due to complex actions of different inflammatory cytokines and the subsequent hemodynamic decompensation. This type of sepsis with impairment of vital organs is referred to as severe sepsis, and the condition of sepsis-induced hypotension persisting despite adequate fluid resuscitation is referred to as septic shock, and mortality for these cases of sepsis are higher than in general sepsis [14].

In the treatment of sepsis, the goal of resuscitation is to increase oxygen, restore intravascular volume, and reverse organ dysfunction and compliance with delivering the sepsis treatment bundle (such as measurement of lactate level at early stage of diagnosis, blood culture tests, fluid resuscitation, administration of broad-spectrum antibiotics, and vasopressor therapy) has been reported to be crucial in increasing the survival rate of the patients [15]. However, it has been reported that compliance with delivering the treatment bundle did not have a significant impact on mortality [16]. Development of adequate therapies for sepsis is required.

BJT is a herbal prescription mainly consisting of the following medicinal plants: Astragali radix (Huang Qi) at 1.5 don (1 don; 3.75g), Ginseng radix (Ren Shen), Atractyloides Rhizome White (Bai Zhu), and Glycyrrhizae radix (Gan Cao) at 1 don, respectively, Angelicae radix (Dangguishen) and Citrus Unshiu Peel (Chen Pi) at 5 pun (1 pun; 0.375g), respectively, and Cimicifugae rhizome (Sheng Ma) and Bupleuri radix (Cai Hu) at 3 pun, respectively. The prescription originated from the Pi-Wei theory (Theory on Spleen and Stomach) of Li Dongyuan in the Jin Yuan Dynasty [17]. BJT has an anti-inflammatory effect and it has been reported in an in vitro study using RAW264.7 murine macrophages where LPS and interferon gamma induced inflammation, the expression of nitric oxide, prostaglandin E2, iNOS, COX-2 and inflammatory cytokines were lower when BJT was used as a pretreatment prior to inducing inflammation as compared with LPS and interferon gamma treatment alone [18]. A study on the regulation of LPS-induced nitric oxide, TNF-α, and IL-6 expression levels in RAW264.7 macrophages studied the differences in Korean, Chinese, and Japanese prescriptions of BJT [19]. In addition, studies reported its anti-allergic effect in murine models [20,21]. Moreover, recent studies have demonstrated the anti-inflammatory effects of the herbal components of BJT, including Astragali radix [22], Ginseng radix [23], Atractyloides Rhizome white [24], Glycyrrhizae radix [25], Angelicae radix [26], Citrus Unshiu Peel [27], and Bupleuri radix [28]. Therefore, we can reasonably expect that BJT will be effective in the treatment of systemic inflammatory responses such as sepsis. However, to date, there has been no study that investigated the effect of BJT on improvement of sepsis. Therefore, this study investigated the effect of BJT on the inflammatory responses of LPS-treated RAW264.7 macrophages and related symptoms in a septic mouse model using intraperitoneal injection of LPS.

Sepsis-induced ALI occurs due to severe inflammatory responses in the respiratory system [29], which may eventually lead to respiratory failure and death of the patients [30]. In this study, the effect of BJT on sepsis was investigated, especially focusing on the effect on lung injury. In a mouse model with intraperitoneal injection of LPS in which sepsis and the consequent ALI are induced, pretreatment with BJT prior to LPS treatment showed less infiltration of inflammatory cells into lung tissue compared with LPS alone. In addition, ALI symptoms such as bleeding from the pulmonary microvasculature into the alveoli, interstitial thickening, and edema were less severe when BJT was administered prior to LPS treatment compared with LPS treatment. Moreover, using the dry/wet ratio, which is an index for evaluation of fibrosis and edema in lung tissue [31], the effect of BJT pretreatment resulted in a less severe response to LPS induced sepsis and smaller edema in the lung tissue.

Cytokines are regulators of the immune response against infection or endotoxins, and in particular, pro-inflammatory cytokines stimulate inflammation and regulate early immune responses [32]. IL-4 is a cytokine regulating the response of T helper cells (Th1 and Th2), and is closely related to the suppression of cell-mediated immunity and to sepsis-related deaths [33], and has been reported as a predictor for the prognosis of patients with sepsis [34]. IL-6 is also a pro-inflammatory cytokine playing a crucial role in immune-mediated inflammatory diseases. It is secreted from various cell types including T cells and macrophages and has been reported to be highly associated with the development of sepsis [35]. Among different types of cytokines, a high level of IL-6 in the serum has been reported to show the highest correlation with sepsis-induced mortality [36]. IL-8 is well known as a cytokine that induces activation of neutrophils [37]. IL-8 levels have been reported to be increased in most patients with sepsis [38] and it has been reported as a cytokine that can be used to monitor the prognosis of the patients [39] or as a therapeutic target for acute condition of inflammatory responses such as sepsis [40]. TNF-α is a pleiotropic cytokine that plays an important role in multiple immune-mediated inflammatory diseases. Cytokines mediate various types of cell activities such as proliferation, survival, differentiation, and cell death, and cause the main symptoms of sepsis such as fever, hemodynamic abnormalities, loss of appetite, joint pain, and local accumulation of neutrophils [41]. The in vivo model with intraperitoneal administration of LPS in C57BL/6 mice induced a significant increase in the expression of these cytokines, and serum IL-6 and TNF-α levels were significantly lower when there was pretreatment with BJT. In the in vitro model, BJT treatment of RAW264.7 macrophages prior to LPS treatment had an inhibitory effect compared with LPS treatment alone on the expression of
mRNA levels and secretion of inflammatory cytokines such as IL-6 and TNF-α.

Various factors are involved in the development of sepsis. TLR4 acts as a receptor for LPS on the cell wall of the bacterial pathogen [42]. TLR4 signaling pathway plays an important role in immune responses through the regulation of mitochondrial reactive oxygen species in macrophage bactericidal activity [43], and TLR4-deficient mice have been reported to be hyporesponsive to LPS-induced sepsis [44]. COX-2 is an enzyme responsible for the formation of inflammatory prostanooids such as prostaglandins, and since COX-2 is one of the downstream products of TLR4 activation, COX-2 may show TLR4 dependent signaling in the pathophysiology of sepsis [45]. Research using an animal model of sepsis reported the effectiveness of COX inhibition and potential use as a treatment for sepsis [46]. LPS and inflammatory cytokines also cause an increase in iNOS, and when this is accompanied by hyperproduction of nitric oxide, it may lead to a pathological dilatation of conduit arteries, leading to hypotension and heart failure in patients with sepsis [47]. In RAW264.7 macrophages in this study, pretreatment with BJT was effective in suppressing the LPS-induced increases in iNOS, COX-2, and TLR4, and it can be reasoned that BJT may contribute to alleviating LPS-stimulated septic conditions and severe immune responses.

NFκB plays a critical role in all inflammatory responses, including innate and adaptive immunity by regulating the activity of various immune cells and the production of inflammatory factors [48]. NFκB activation has been reported to be associated with the pathological mechanism of sepsis [49]. In particular, NFκB has been reported to contribute to infiltration of neutrophils to multiple organs [50] and be involved in the overactivation of endothelial nitric oxide synthase [51]. MAPK signaling pathways have also been reported to play an important role in inflammatory responses [52]. Since MAPK is also involved in the pathogenesis of sepsis, MAPK can be used as a therapeutic target along with NFκB [53], or on its own [54,55]. BJT treatment of RAW264.7 macrophages prior to LPS treatment, suppressed the activation of MAPK mechanisms (such as reducing the phosphorylation of MAPKs including extracellular signal-regulated kinase, Jun N-terminal kinase, and p38), and also regulated the activation of NFκB (which acts as a key factor in inflammatory responses) when compared with LPS treatment alone.

This study examined the in vivo effect of BJT treatment given to mice prior to LPS-induced sepsis and the subsequent ALI, and in vitro in LPS-treated RAW264.7 macrophages. BJT treatment prior to LPS treatment suppressed cell proliferation, inflammatory cytokine levels, and pro-inflammatory mediators and related factors when compared with LPS treatment alone in vitro. In vivo, as a result of oral administration of BJT for 2 weeks prior to induction of sepsis, the LPS-induced pathological changes such as bleeding from the pulmonary microvasculature into the alveoli, interstitial thickening and edema as well as immune cell infiltration observed in lung tissue were less severe, and the expression of inflammatory cytokines in serum was significantly lower compared with LPS treatment alone. The findings of this study indicated that BJT may be an effective therapeutic agent for sepsis and sepsis-induced ALI, both of which do not have proper treatments currently available.

Author Contributions

Conceived and designed the study: JP. Performed the experiments and analyzed the data: HIK and YH. Wrote the initial manuscript: HIK and YH. Revised and edited the manuscript: JP

Conflicts of Interest

The authors declare no conflicts of interest.

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Ethics Statement

All animal experiments were conducted in accordance with the Declaration of Helsinki, and according to a protocol approved by the Animal Care and Use Committee of the Institutional Review Board of Kyung Hee University (confirmation no.: KHSASP-22-131).

Data Availability

All relevant data are included in this manuscript.

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