Prev. Nutr. Food Sci. 2019;24(3):344-347
https://doi.org/10.3746/pnf.2019.24.3.344
pISSN 2287-1098 · eISSN 2287-8602

Note

Pandanus fascicularis Lam Extract Inhibits Pro-Inflammatory Cytokines Production in LPS-Stimulated RAW 264.7 Cells

Sun-Yup Shim

Department of Aqualife Science, College of Fisheries and Ocean Sciences, Chonnam National University, Jeonnam 59626, Korea

ABSTRACT: *Pandanus fascicularis* Lam has been shown to exert to a variety of physiological effects on edema, tumors, leprosy, spasm, inflammation, pain, and rheumatoid arthritis. In this study, the effects of a *P. fascicularis* extract on the production of lipopolysaccharide (LPS)-induced pro-inflammatory cytokines interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α expression and on the production of the inflammatory mediator, nitric oxide (NO), in RAW 264.7 cells were examined. The *P. fascicularis* extract decreased LPS-induced NO production. Moreover, enzyme-linked immunosorbent assays revealed that the *P. fascicularis* extract concentration-dependently suppressed LPS-induced production of the pro-inflammatory cytokines IL-1β, IL-6, and TNF-α. These results suggest that the *P. fascicularis* extract has potential as an anti-inflammatory therapeutic substance for use in the prevention of the inflammatory disorder.

Keywords: *Pandanus fascicularis* Lam, IL-1β, IL-6, TNF-α, RAW 264.7 cells

INTRODUCTION

The inflammatory response is an important host defense-system reaction to injury, autoimmune disease, and tissue ischemia and is typically characterized by redness, swelling, heat, and pain. Among the cell types involved in the inflammatory response, macrophages have a pivotal role and act as major inflammatory and immune effector cells (Serhan and Savill, 2005). Macrophages are activated by stimulants such as cytokines, chemokines, and bacterial lipopolysaccharide (LPS) (Pierce, 1990). LPS, the major component of the cell wall of Gram-negative bacteria, is associated with a wide variety of inflammatory diseases and has been widely used to mimic the features of the inflammatory disorder (Ko et al., 2019; Laveti et al., 2013; Ham et al., 2017). LPS-activated macrophages produce a variety of pro-inflammatory mediators such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, as well as nitric oxide (NO) (Boscá et al., 2005; Kim et al., 2013; Xie and Nathan, 1993). Excessive and aberrant production of these cytokines or NO can lead to the pathogenesis of chronic diseases including atherosclerosis, cancer, and inflammatory arthritis (Boscá et al., 2005; Medzhitov and Janeway, 1997; Serhan and Savill, 2005; Vezza et al., 2016). Thus, downregulation of the production of these substances would be attractive in the development of potential therapeutic applications for the treatment of inflammatory diseases (Ahmed, 2011; Guha and Mackman, 2001).

Pandanus fascicularis Lam, referred to as screw pine, is a palm-like evergreen tree or shrub of the Pandanaceae family. *P. fascicularis* is distributed mainly in subtropical and tropical regions, is native to South Asia, and, in particular, has a significant presence in mangrove swamps (Loa et al., 2016; Vinod et al., 2007; Vinod et al., 2010). The leaves of *P. fascicularis* are useful in edema, tumor, leprosy, antispasmodic, inflammation, pain, and rheumatoid arthritis applications (Panda et al., 2008; Rajeswari et al., 2011). However, the inhibitory effects of *P. fascicularis* Lam on pro-inflammatory cytokines production have not yet been examined. In the present study, LPS-stimulated RAW 264.7 cells were treated with *P. fascicularis* extract to determine whether a *P. fascicularis* extract has inhibitory effects on pro-inflammatory cytokines.

MATERIALS AND METHODS

Chemicals

Dulbecco's modified Eagle's medium (DMEM), penicillin and streptomycin solution, and fetal bovine serum (FBS) were obtained from Hyclone Laboratories, Inc. (Logan,

Received 18 February 2019; Accepted 13 May 2019; Published online 30 September 2019

Correspondence to Sun-Yup Shim, Tel: +82-61-659-7160, E-mail: ssmami@chonnam.ac.kr
Author information: Sun-Yup Shim (Researcher)

UT, USA). The CellTiter 96® Aqueous One Solution Cell Proliferation Assay kit was obtained from Promega (Madison, WI, USA). The enzyme-linked immunosorbent assay (ELISA) kits for cytokines was obtained from BD OptEIA™ (San Diego, CA, USA).

Preparation of extract

The dried leaves of *P. fascicularis* Lam were purchased from the "gourmet market" in Bangkok, Thailand. For extraction, 10 volumes of 70% ethanol were added to the powered *P. fascicularis* Lam leaves. The supernatant of the mixture was condensed in a vacuum and lyophilized. The *P. fascicularis* Lam extract was stored at −20°C and dissolved in dimethyl sulfoxide before the use.

Cell culture, treatment, and stimulation

The murine macrophage, RAW 264.7 cells were obtained from the Korean Cell Line Bank (Seoul, Korea) and were maintained in DMEM supplemented with 10% heat-inactivated FBS and penicillin and streptomycin solution. The cells were cultured at 37°C in a humidified atmosphere with 5% CO_2. The cells were cultured in serum-free DMEM medium with various concentrations of *P. fascicularis* Lam extract for 1 h, and then stimulated with 1 μg/mL of LPS for 24 h. All experiments were conducted by 1 μg/mL of LPS for the induction of inflammation.

Cell viability assay

RAW 264.7 cells (1×10^5 cells/well) were plated in 96-well plates and incubated overnight, pre-treated with *P. fascicularis* Lam extract for 1 h and then stimulated with LPS for 24 h in the presence of extract. The cytotoxic effect was evaluated by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS, Promega, Madison, WI, USA) assay according to manufacturer's instruction. Briefly, the cells were removed medium and the reagent of 10 times diluted with fresh medium was added to each well, and incubated for 1 h and measured at 490 nm.

Measurement of NO production

The cells were treated with *P. fascicularis* Lam extract for 1 h, and induced by stimulating them with LPS for 24 h. The culture media was mixed with an equal amount of Griess reagent, reacted at room temperature for 15 min, and measured at 550 nm using a microplate reader (Bio-Tek Instruments, Inc., Winooski, VT, USA). Serum free culture medium was used as the blank in all experiments.

Cytokine production

The culture supernatant of the treated and stimulated cells (2.5×10^5 cells/well) were collected for determination of the pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α by ELISA kit according to manufacturer's instruction.

Statistical analysis

Data are expressed as means±standard deviation (SD) of at least three independent experiments. One-way ANOVA was used for comparisons of multiple group means followed by the Student's *t*-test and statistical significance was considered at $P<0.05$.

RESULTS AND DISCUSSION

Toxicity effect on RAW 264.7 cells

P. fascicularis has been shown to exert to a variety of physiological effects on edema, tumors, leprosy, spasm, inflammation, pain, and rheumatoid arthritis. Macrophages have a pivotal role in the innate immune response as an effector cell, and they are the major cellular targets of LPS (Pierce, 1990; Vezza et al., 2016). LPS is a potent stimulant known to induce an inflammatory response (Ko et al., 2019; Laveti et al., 2013; Ham et al., 2017). The cytotoxic effect of the *P. fascicularis* extract on RAW 264.7 cells was assessed by using MTS assays. RAW 264.7 cells were treated with different concentrations of the *P. fascicularis* extract for 24 h and then stimulated by adding LPS. As shown in Fig. 1, the *P. fascicularis* extract did not have a cytotoxic effect on RAW 264.7 cells treated for 24 h at extract concentrations up to 100 μg/mL.

Effects on LPS-induced NO production

NO is an important molecule that regulates physiological and pathological processes in the inflammatory response. To examine the effect of *P. fascicularis* extract on LPS-in-

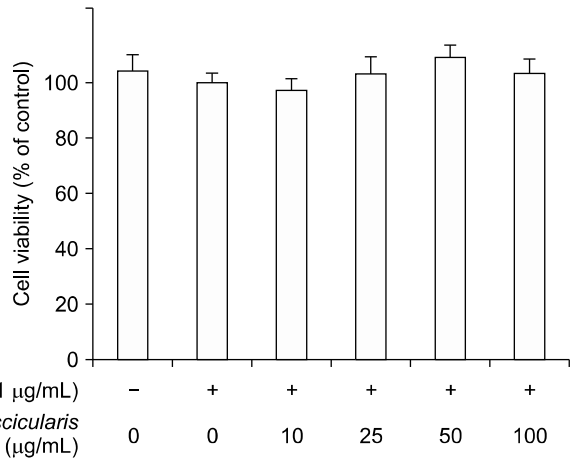

Fig. 1. Effects of *Pandanus fascicularis* Lam extract on cytotoxicity in lipopolysaccharide (LPS)-induced RAW 264.7 cells. Cells were pretreated with *P. fascicularis* Lam extract for 1 h and then stimulated with LPS (1 mg/mL) for 24 h under serum-free conditions. Cell viability were determined using MTS assay. Each determination was made in triplicate. Each determination was made in triplicates. Data are presented as mean±SD.

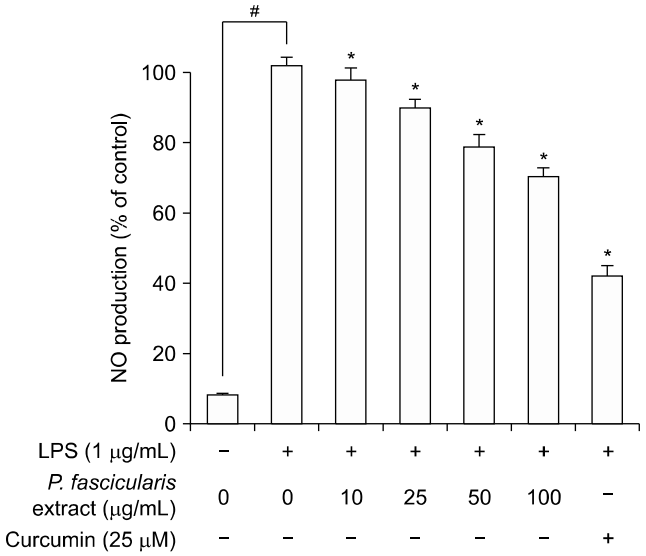

Fig. 2. Effects of *Pandanus fascicularis* Lam extract on nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW 264.7. Cells were pretreated with *P. fascicularis* Lam extract for 1 h and then stimulated with LPS (1 mg/mL) for 24 h under serum-free conditions. NO production was determined using Griess assay. Each determination was made in triplicates. Data are presented as mean±SD. $^{\#}P<0.05$ vs. control and $^{*}P<0.05$ vs. LPS-treated group.

duced NO production, cells were pretreated with *P. fascicularis* extract for 1 h followed by stimulation with LPS (1 μg/mL) for 24 h. Curcumin was used as a positive control treatment. The LPS-induced NO production was measured by using Griess reagent. The results showed that, compared to the vehicle control treatment, NO production was markedly induced by LPS stimulation. However, pre-treatment with the *P. fascicularis* extract significantly decreased the level of LPS-induced NO production in a concentration-dependent manner (Fig. 2).

P. fascicularis extract inhibits LPS-induced pro-inflammatory cytokine production

Pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) are recognized as key markers of inflammation and are secreted during an early stage of the inflammatory response (Medzhitov and Horng, 2009; Turner et al., 2014). To assess the anti-inflammatory activity of the *P. fascicularis* extract on inflammatory signaling in LPS-induced macrophages, LPS-induced IL-1β, IL-6, and TNF-α cytokine production levels were measured by performing ELISA. Treatment with the *P. fascicularis* extract (10～100 μg/mL) concentration-dependently suppressed IL-1β, IL-6, and TNF-α productions in LPS-stimulated RAW 264.7 cells (Fig. 3). These results demonstrate that the *P. fascicularis* extract is a promising novel natural therapeutic material for use in the treatment of inflammatory disorders. Further studies on the protective effects of the active compounds in the *P. fascicularis* extract are necessary to confirm the potential therapeutic application of *P. fascicularis* in the treatment of inflammatory diseases.

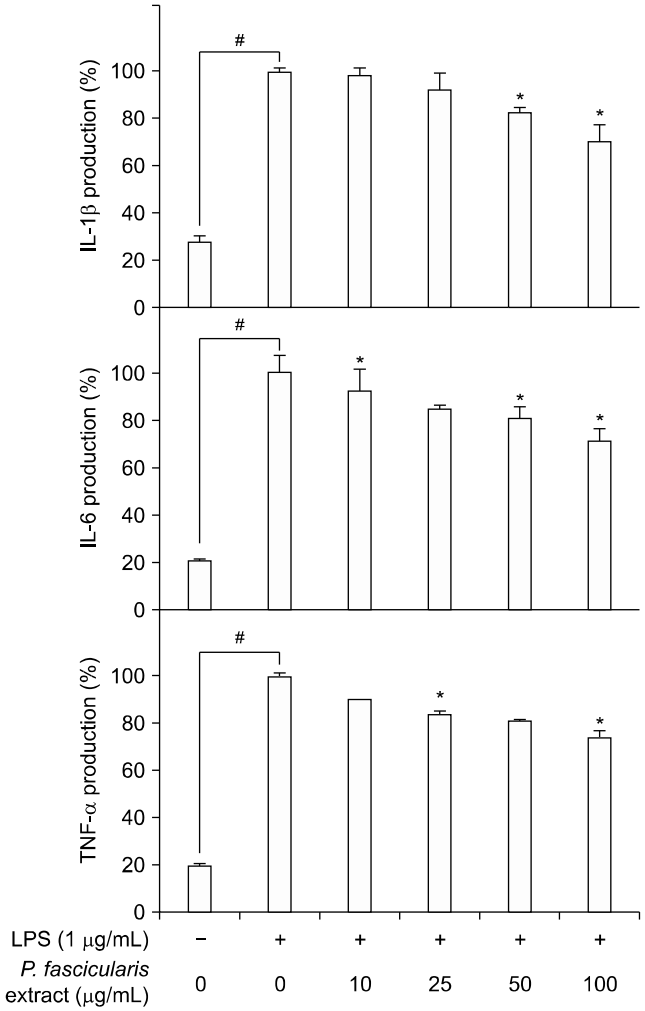

Fig. 3. Effects of *Pandanus fascicularis* Lam extract on production of pro-inflammatory cytokines. Cells were pretreated with *P. fascicularis* Lam extract for 1 h and then stimulated with lipopolysaccharide (LPS, 1 μg/mL) for 24 h. Levels of interleukin (IL)-1β (A), IL-6 (B), and tumor necrosis factor (TNF)-α (C) in culture media were determined by ELISA according to manufacturer's insutruction. Each determination was made in triplicates. Data are presented as mean±SD. $^{\#}P<0.05$ vs. control and $^{*}P<0.05$ vs. LPS-treated group.

AUTHOR DISCLOSURE STATEMENT

The author declares no conflict of interest.

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