Anti-Inflammatory Effect of Fermented *Liriope platyphylla* Extract in LPS-stimulated RAW 264.7 Macrophages

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Abstract

The present study was designed to evaluate the inhibitory effects of fermented *Liriope platyphylla* extract on the production of inflammation-related mediators (NO, ROS, NF-κB, iNOS and COX-2) and pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) in lipopolysaccharide-stimulated RAW 264.7 macrophages. Freeze-dried *Liriope platyphylla* was fermented by *Saccharomyces cerevisiae* and extracted with 70% ethanol. In lipopolysaccharide-stimulated macrophage cells, the treatment with fermented *Liriope platyphylla* extract decreased the generation of intracellular reactive oxygen species dose-dependently and increased antioxidant enzyme activities, including superoxide dismutase, catalase and glutathione peroxidase. Fermented *Liriope platyphylla* extract also inhibited NO production in lipopolysaccharide-stimulated RAW 264.7 cell. The expressions of NF-κB, iNOS, COX-2 and pro-inflammatory cytokines were inhibited by the treatment with fermented *Liriope platyphylla* extract. Thus, this study shows the fermented *Liriope platyphylla* extract could be effective at inhibiting the inflammation process.

Key words: fermented *Liriope platyphylla*, anti-inflammation, lipopolysaccharide, RAW 264.7 cell

INTRODUCTION

Inflammation is associated with several human pathologies and causes the up-regulation of several inflammatory mediators such as cytokines. Cytokines, which are produced and secreted by macrophages, play a major role in the induction and regulation of inflammatory cellular interaction (1). Lipopolysaccharides (LPS) produce reactive oxygen species (ROS), such as superoxide anion radical, hydroxyl radical and nitric oxide (NO), and can also induce strong pro-inflammatory mediators such as interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α, inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX)-2 in macrophages (2,3). Overproduction of NO induced by LPS through iNOS can reflect the degree of inflammation, and iNOS induction provides a means of assessing the effect of agents on the inflammatory process (4).

The ROS are involved in inflammatory gene expression by causing redox-based activation of nuclear factor-kappa B (NF-κB) and the COX-2 signaling pathway (5). The production of inflammatory mediators is primarily regulated at the level of gene transcription through the activities of several transcription factors such as NF-κB. Thus, activated NF-κB translocates to the nucleus and then binds to its cognate DNA binding site in the promoter or enhancer regions of specific genes, and induces the transcription of pro-inflammatory media-

tors, *e.g.*, iNOS, COX-2, IL-1β, IL-6 and TNF-α (6).

Plant-derived phytochemicals are an important and promising group of potential anti-inflammatory agents because of their low toxicity and apparent benefit in acute and chronic diseases (7). *Liriope platyphylla* has been widely used in oriental medicine as a tonic, antitussive and expectorant for the treatment of various diseases (8). In addition, *Liriope platyphylla* has also been used to treat diabetes and cardiovascular disease and as an anti-inflammatory agent (9,10).

The compounds of *Liriope platyphylla* have been identified as homoisoflavonoids (ophiopogone, methylophiopoginanone), sterols (β-sistosterol) and steroidal glycosides (ruscogenin, ophiopogonine) (11). Fermentation is a biochemical reaction that metabolizes high-molecular weight organic compounds yielding relatively simple products. The compounds in *Liriope platyphylla* may be metabolized or changed with the compounds having high antioxidant activity by fermentation. Thus, this study examined the effect of fermented *Liriope platyphylla* extracts (FLPE) on antioxidant activities and evaluated the anti-inflammatory effect of FLPE in LPS-stimulated RAW 264.7 macrophages.

MATERIALS AND METHODS

Preparation of FLPE

Liriope platyphylla was purchased from Miryang,

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Korea. *Liriope platyphylla* was roasted at $120^{\circ}C$ for 15 min and then ground for 200 mesh. Ground *Liriope platyphylla* was extracted 3 times with 70% ethanol. For fermentation of *Liriope platyphylla*, *Saccharomyces cerevisiae* obtained from Korean Culture Center of Microorganisms (Seoul, Korea) was inoculated into YM broth and incubated at $30^{\circ}C$ for 24 hr. After the pure culture was obtained, the mycelia were reinoculated with YM broth at $30^{\circ}C$ for 2 days. The mycelia and broth were blended together, and the mixture was added at 1% to the ground *Liriope platyphylla* with water for further cultivation at $30^{\circ}C$. At 3 days, fermented *Liriope platyphylla* was harvested and extracted 3 times with 70% ethanol. Then the extracts were filtered, the ethanol was removed under reduced pressure by rotary evaporator and the extracts were concentrated under vacuum.

DPPH radical scavenging activity

The scavenging activity of fermented *Liriope platyphylla* extracts (FLPE) and *Liriope platyphylla* extracts (LPE) on DPPH radical was studied, employing the modified method describe by Yamaguchi et al. (12); 1.5 mL of DPPH solution (0.1 mmol, in 95% ethanol) was incubated with varying concentrations of the sample dissolved in ethanol. The reaction mixture was shaken and incubated for the resulting solution. The absorbance of the solution was read at 517 nm against blank.

Superoxide anion radical scavenging activity

The scavenging activity for superoxide anion radical was analyzed via a hypoxanthine/xanthine oxidase generating system coupled with nitroblue tetrazolium (NBT) reduction following the method of Kirby and Schmidt (13). The reaction mixture contained 125 μL of buffer (50 mmol K_2HPO_4/KOH, pH 7.4), 20 μL of 15 mmol Na_2EDTA in buffer, 30 μL of 3 Mm hypoxanthine in buffer, 50 μL of xanthine oxidase in buffer (1 unit per 10 mL buffer) and 25 μL of plant extract in buffer (a diluted sonicated solution of 10 μg per 250 μL buffer). The absorbance of the solution was measured at 540 nm.

Hydroxyl radical scavenging activity

The reaction mixture contained 0.45 mL of 0.2 M sodium phosphate buffer (pH 7.0), 0.15 mL of 10 mmol 2-deoxyribose, 0.15 mL of 10 mmol $FeSO_4$-EDTA, 0.15 mL of 10 mmol H_2O_2, 0.525 mL of H_2O and 0.075 mL of sample solution. The reaction was started by the addition of H_2O_2. After incubation at $37^{\circ}C$ for 4 hr, the reaction was stopped by adding 0.75 mL of 2.8% trichloroacetic acid and 0.75 mL of 1.0% 2-tribarbituric acid in 50 mmol NaOH. The solution was boiled 10 min and then cooled in water. The absorbance of the solution was measured at 520 nm. Hydroxyl radical scavenging activity was evaluated as the inhibition rate of 2-deoxyribose oxidation by $\cdot OH$ (14).

Antioxidant activity on linoleic acid oxidation

The oxidation test was conducted by using the linoleic acid model system. A 0.2 mL of sample solution and 0.5 mL of 0.2 M sodium phosphate buffer (pH 7.0) were mixed with 0.5 mL of 2.5% linoleic acid in ethanol. The peroxidation was initiated by the addition of 50 μL of 0.1 M 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) and carried out at $37^{\circ}C$ for 200 min in the dark. The degree of oxidization was measured according to the thiocyanate method (15) for measuring peroxides by reading the absorbance at 500 nm after coloring with $FeCl_2$ and ammonium thiocyanate. A control test was performed with linoleic acid without sample solution.

Cell culture and treatment

Mouse macrophage RAW 264.7 cells were cultured in Dulbecco's modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 100 U/mL of penicillin and 100 μg/mL of streptomycin at $37^{\circ}C$ in 5% CO_2/95% air. Cells in 96 well plates (2×10^4 cells/well) were treated with FLPE (0.05, 0.1, 0.25, 0.5 mg/mL) for 2 hr, and then incubated with LPS (1 μg/mL) for 20 hr.

Cell viability

Cell viability was assessed using a modified MTT assay (16). Briefly, cells (2×10^4 cells/well) were seeded in a 96 well plate and treated with FLPE. Following treatment, 100 μL of an MTT solution (5 mg/mL in phosphate buffered saline) was added to each well and further incubated for 4 hr at $37^{\circ}C$. Subsequently, 100 μL of dimethyl sulfoxide (DMSO) was added to each well to dissolve any deposited formazon. The optical density (OD) of each well was measured at 540 nm with a microplate reader.

Measurement of intracellular ROS level

Intracellular ROS levels were measured by the 2',7'-dichlorofluorescein diacetate (DCF-DA) assay (17). DCF-DA can be deacetylated in cells, where it can react quantitatively with intracellular radicals to convert into its fluorescent product, DCF, which is retained within the cells. Therefore, DCF-DA was used to evaluate the generation of ROS in oxidative stress. Cells (2×10^4 cells/well) were seeded in a 96 well plate and pre-incubated with various concentrations (0.05, 0.1, 0.25 and 0.5 mg/mL) of FLPE for 2 hr in humidified atmosphere containing 5% CO_2 at $37^{\circ}C$. After 2 hr of incubation, the cells were incubated with LPS (1 μg/mL) for 20 hr. Thereafter, the medium was removed and the cells were washed twice with phosphate buffered saline (PBS, pH 7.4) and

then were incubated with 5 μm DCF-DA for 30 min at room temperature. Fluorescence was measured using a fluorescence plate reader.

Measurement of antioxidant enzyme activities

Cells (2×10^4 cells/well) in a 96 well plate were pre-incubated with various concentrations (0.05, 0.1, 0.25 and 0.5 mg/ mL) of FLPE for 2 hr, then further incubated with LPS for 20 hr. The medium was removed and the cells were washed twice with PBS. One mL of 50 mmol/L potassium phosphate buffer with 1 mmol/L EDTA (pH 7.0) was added and the cells were scraped. Cell suspensions were sonicated three times for 5 sec on ice each time, then cell sonicates were centrifuged at $10,000 \times g$ for 20 min at 4°C. Cell supernatants were used for measuring antioxidant enzyme activities. The protein concentration was measured by using the method of Bradford (18) with bovine serum albumin as the standard. SOD activity was determined by monitoring the auto-oxidation of pyrogallol. A unit of SOD activity was defined as the amount of enzyme that inhibited the rate of oxidation of pyrogallol. Catalase activity was measured according to method of Aebi (19) by following the decreased absorbance of H_2O_2. The decrease of absorbance at 240 nm was measured for 2 min. Standards containing 0, 0.2, 0.5, 1 and 2 mmol/L of H_2O_2 were used for the standard curve. GSH-px activity was measured by using the method of Lawrence and Burk (20). One unit of GSH-px is defined as the amount of enzyme that oxidized 1 nmol of NADPH consumed per minute.

Measurement of NO level

Cells (2×10^4 cells/well) were seeded in a 96 well plate and pre-incubated with the indicated concentrations of FLPE in humidified atmosphere containing 5% CO_2 at 37°C for 2 hr. After 2 hr of incubation, the cells were incubated with LPS (1 μg/mL) for 20 hr. Thereafter, each 50 μL of culture supernatant was mixed with an equal volume of Griess reagent [0.1% N-(1-naphthyl)-ethyl-enediamine, 1% sulfanilamide in 5% phosphoric acid] and incubated at room temperature for 10 min, The absorbance at 550 nm was measured in a microplate absorbance reader, and a series of known concentration of sodium nitrite was used as a standard (21).

Western blotting

TNF-α, IL-1β, IL-6, iNOS and COX- 2 expression and NF-κB DNA binding activity were determined by Western blot analysis. Total protein for TNF-α, IL-1β, IL-6, iNOS, COX-2 protein levels and nuclear protein for NF-κB were electrophoresed through 10% sodium dodecyl sulfate-polyacrylamide gel. Separated proteins were transferred electrophoretically to a pure nitrocellulose membrane, blocked with 5% skim milk solution for 1 hr, and

then incubated with primary antibodies overnight at 4°C (22). After washing, the blots were incubated with goat anti-rabbit or goat anti-mouse IgG HRP conjugated secondary antibody for 1 hr at room temperature. Each antigen-antibody complex was visualized using ECL western blotting detection reagents and detected by chemiluminescence with LAS-1000 plus. Band densities were determined by an image analyzer ATTO densitograph and normalized to β-actin for total protein and nuclear protein.

Statistical analysis

The data were represented as mean±SD. The statistical analysis was performed using SAS software. The values were evaluated by one-way analysis of variance (ANOVA) followed by post-hoc Duncan's multiple range tests.

RESULTS AND DISCUSSION

Radical scavenging activities of FLPE

The IC_{50} value of radical scavenging and antioxidant activities of FLPE and LPE are shown in Table 1. FLPE had significantly lower IC_{50} value of DPPH radical scavenging activity (0.18 ± 0.06 mg/mL) than that of LPE (0.49 ± 0.01 mg/mL). DPPH is a free radical donor, which has been widely used to evaluate the free radical scavenging effect of natural antioxidants. Superoxide anion is formed in viable cells during several biochemical reactions and its effect can be magnified because it produces other types of free radicals and oxidizing agent that can induce cell damage (23). FLPE indicated significantly lower IC_{50} value of superoxide anion radical scavenging activity (0.19 ± 0.04 mg/mL) than that of LPE (1.23 ± 0.03 mg/mL). The IC_{50} value of hydroxyl radical scavenging activity of FLPE also was 0.90 ± 0.19 mg/mL, which was lower than that of LPE. The cell-damaging action of hydroxyl radical is well known, as it is the

Table 1. IC_{50} value[1] of radical scavenging and antioxidant activities of fermented *Liriope platyphylla* extracts

	IC_{50} (mg/mL)	
	FLPE	LPE
DPPH radical scavenging	0.18 ± 0.06**	0.49 ± 0.01
Superoxide anion radical scavenging	0.19 ± 0.04**	1.23 ± 0.03
Hydroxyl radical scavenging	0.90 ± 0.19**	2.18 ± 0.10
Antioxidant activity on linoleic acid oxidation	0.36 ± 0.12*	0.50 ± 0.07

[1]IC_{50} value is the concentration of sample required for 50% inhibition. Each value is expressed as mean±SD (n=3). *$p < 0.05$, **$p < 0.01$ by *t*-test. FLPE: fermented *Liriope platyphylla* extracts with *Saccharomyces cerevisiae*, LPE: *Liriope platyphylla* extracts.

strongest among free radicals (24). The antioxidant activity of FLPE on the peroxidation of linoleic acid was investigated. The IC_{50} value of the anti-lipid peroxidation effect of FLPE was 0.36 ± 0.12 mg/mL in comparison to that of LPE (0.50 ± 0.07 mg/mL). In this study, FLPE increased free radical scavenging and antioxidant activities in comparison with LPE.

Fermentation is a chemical reaction that splits complex organic compounds into relatively simple substances. During fermentation, the compounds in *Liriope platyphylla* might be metabolized and the metabolites may have effect on antioxidants activities. Many similar studies have been reported about the correlation between fermentation and free radical scavenging activities and having higher antioxidant activity by fermentation (25,26). Free radicals may injure cells and tissue directly via oxidative degradation of essential cellular components. It is becoming increasingly apparent that reactive oxygen metabolites may initiate and/or amplify inflammation via the upregulation of several different genes involved in the inflammatory response, such as those that code for pro-inflammatory cytokines (27). As described above, FLPE showed greater radical scavenging and antioxidant activity than LPE, thus, we investigated the anti-inflammatory effect of FLPE in LPS-stimulated RAW 264.7 cells.

Cell viability

RAW 264.7 cell viability in the presence of LPE and FLPE are shown in Fig. 1. The result showed that LPE and FLPE at the concentrations of $0 \sim 1$ mg/mL had no effect on the viability and were not toxic to RAW 264.7 cell.
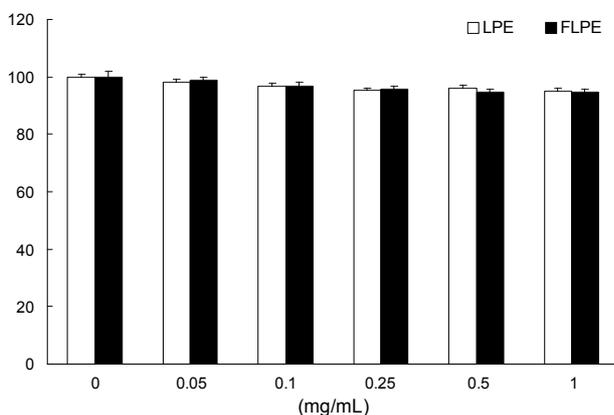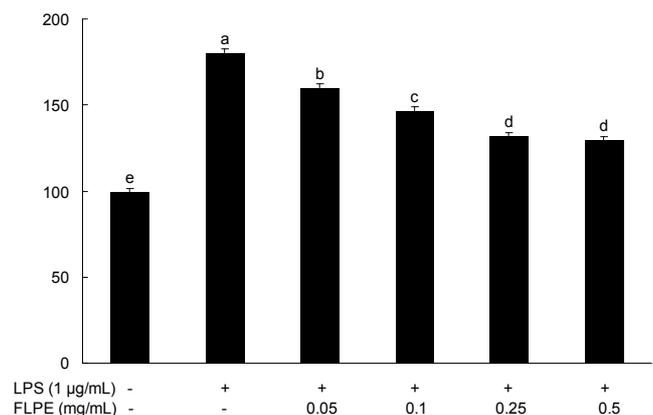

Fig. 1. Effects of LPE and FLPE on viability in LPS-stimulated RAW 264.7 cells. Cells (2×10^4 cells/well) in 96-well plates were first incubated with and without indicated concentrations of FLPE for 20 hr. Each value is expressed as mean $\pm$ SD in triplicate experiments. LPE: *Liriope platyphylla* extract, FLPE: fermented *Liriope platyphylla* with *Saccharomyces cerevisiae* extract.

Intracellular ROS generation

ROS are commonly produced during inflammatory processes and play an important role in host defense against infection (28). In RAW 264.7 cells, LPS significantly increased ROS generation. In the presence of LPS, FLPE significantly reduced ROS generation in a concentration-defendant manner from 0.05 to 0.25 mg/mL (Fig. 2). The intracellular ROS levels were $160.3 \pm 1.97\%$, $146.6 \pm 2.42\%$, $132.3 \pm 1.52\%$ and $129.7 \pm 2.07\%$ compared with control (with the addition of 0.05, 0.1, 0.25 and 0.5 mg/mL FLPE, respectively). High ROS levels induce oxidative stress and inflammatory reactions, which can result in a variety of biochemical and physiological lesions. ROS have been found to be important mediators that provoke or sustain inflammatory processes and, consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation (29).

Antioxidant enzyme activities

Table 2 shows antioxidant enzyme activities of FLPE in LPS-stimulated RAW 264.7 cells. The activity of SOD was decreased by LPS and recovered by FLPE, with values from 54.01 ± 1.48, 54.78 ± 3.34, 58.24 ± 2.63 and 60.49 ± 1.82 μM/mg protein (0.05, 0.1, 0.25 and 0.5 mg/mL FLPE, respectively). SOD catalyzes the conversion of superoxide anion (O_2^-) to hydrogen peroxide (H_2O_2), and hydrogen peroxide is further reduced to H_2O by the activity of catalase or glutathione peroxidase. SOD may increase hydrogen peroxide that sometimes plays a role as a second messenger in the product of inflammatory cytokines including TNF-α, IL-1β and IL-6 (30). Catalase

Fig. 2. Effects of FLPE on intracellular ROS levels in LPS-stimulated RAW 264.7 cells. Cells (2×10^4 cells/well) in 96-well plates were first incubated with or without indicated concentrations of FLPE for 2 hr, and then incubated with LPS (1 μg/mL) for 20 hr. Untreated is negative control without LPS treatment. Each value is expressed as mean $\pm$ SD in triplicate experiments. [a-e]Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test. FLPE: fermented *Liriope platyphylla* extracts with *Saccharomyces cerevisiae*.

Table 2. Effects of FLPE on antioxidant enzyme activities in LPS-stimulated RAW 264.7 cells

	Untreated	FLPE (mg/mL)+LPS (1 μg/mL)				
		0	0.05	0.1	0.25	0.5
SOD (μM/mg protein/min)	63.07±2.1[a]	38.42±2.4[b]	54.01±1.48[a]	54.78±3.34[a]	58.24±2.63[a]	60.49±1.82[a]
Catalase (μM/mg protein/min)	2.64±0.02[a]	1.50±0.04[f]	1.61±0.04[e]	1.85±0.03[d]	2.08±0.05[c]	2.24±0.02[b]
GSH-px (unit/mg protein)	4.95±0.08[c]	4.07±0.19[e]	4.33±0.07[d]	4.98±0.16[c]	5.30±0.11[b]	5.92±0.09[a]

Cells (2×10^4 cells/well) in 96-well plates were first incubated with or without indicated concentrations of FLPE for 2 hr, and then incubated with lipopolysaccharide (LPS, 1 μg/mL) for 20 hr. Untreated is negative control without LPS treatment. [a-f]Values with different alphabets in a row are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test. SOD: superoxide dismutase, GSH-px: glutathione peroxidase, FLPE: fermented *Liriope platyphylla* extracts with *Saccharomyces cerevisiae*.

activities were significantly increased ($p < 0.05$) by FLPE treatment from 1.61 ± 0.04 to 2.24 ± 0.02 μM/mg protein, with the addition of 0.05 mg/mL FPLE and 0.5 mg/mL FPLE, respectively. Catalase could cause anti-inflammatory effects by destroying hydrogen peroxide and preventing formation of other cytotoxic oxygen species. Also, catalase affects the expression of genes influencing inflammation *in vitro* and *in vivo* (31). Activity of glutathione peroxidase showed a significant increase also, from 4.33 ± 0.07 to 5.92 ± 0.09 unit/mg protein with the addition of 0.05 mg/mL FPLE and 0.5 mg/mL FPLE, respectively. Thus, treatment of LPS-stimulated cells with FLPE enhanced antioxidant enzyme activities that may attenuate inflammation.

NO production

NO production may reflect the degree of inflammation, and provides a measurement to assess the inhibitory effect on the inflammatory process (32). As shown in Fig. 3, the level of NO was significantly increased when
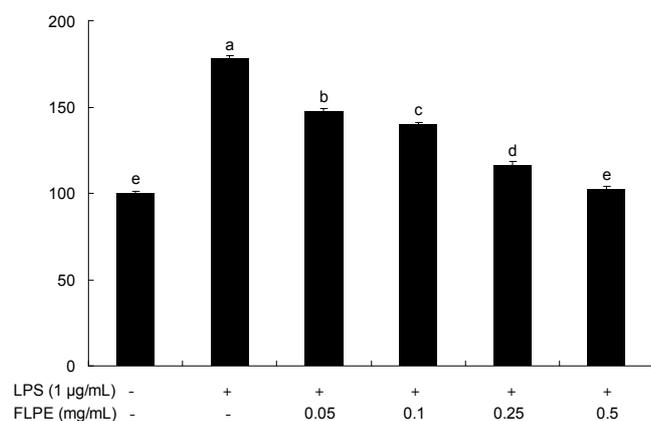
Fig. 3. Effects of FLPE on NO production in LPS-stimulated RAW 264.7 cells. Cells (2×10^4 cells/well) in 96-well plates were first incubated with or without indicated concentrations of FLPE for 2 hr, and then incubated with LPS (1 μg/mL) for 20 hr. Untreated is negative control without LPS treatment. Each value is expressed as mean±SD in triplicate experiments. [a-e]Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test. FLPE: fermented *Liriope platyphylla* extracts with *Saccharomyces cerevisiae*.

stimulated only with LPS. However, NO levels in FLPE treated cells were significantly decreased. The level of NO in LPS-stimulated RAW 264.7 cells was 177.94%, but treatment with 0.05, 0.1, 0.25, 0.5 mg/mL of FLPE resulted in significant decrease in NO levels to 147.13%, 140.05%, 116.04% and 102.21% (with the addition of 0.05, 0.1, 0.25 and 0.5 mg/mL FLPE, respectively). The most prominent phenomenon in the process of inflammation is the increase of NO and pro-inflammatory cytokines. NO is known to act as an intracellular messenger that, in many cases, is involved in promoting inflammatory responses (33,34). Over-production of NO by macrophages may lead to various pathological disorders such as inflammation. Therefore, FLPE could be helpful to the attenuation of inflammatory process with high NO levels.

Effects of FLPE on TNF-α, IL-1β and IL-6 expressions

The expressions of cytokine production (TNF-α, IL-1β and IL-6) in LPS-stimulated RAW 264.7 cells are shown in Fig. 4. In the presence of LPS, the production of pro-inflammatory cytokines was significantly increased. FLPE treatment of the cells significantly decreased the expression of TNF-α, IL-1β and IL-6. TNF-α is a potent activator of macrophages and can stimulate the production or expression of IL-6 and IL-1β (35). IL-6 is well known pro-inflammatory cytokine and regarded as an endogenous mediator of LPS-induced fever. IL-1β is also considered to be a pivotal pro-inflammatory cytokine, primarily released by macrophages (36). The inhibition of pro-inflammatory cytokine is a key mechanism in the control of inflammatory process. In the present study, the production of TNF-α, IL-1β and IL-6 increased when RAW 264.7 cells were stimulated by LPS, but the FLPE treatment of the cells significantly inhibited the production of TNF-α, IL-1β and IL-6. This result indicates that FLPE has an inhibitory effect on the inflammation process.

Effects of FLPE on NF-κB, iNOS and COX-2 expressions

NF-κB was increased in LPS-stimulated RAW 264.7 cells, and the increase was significantly reduced by treat-
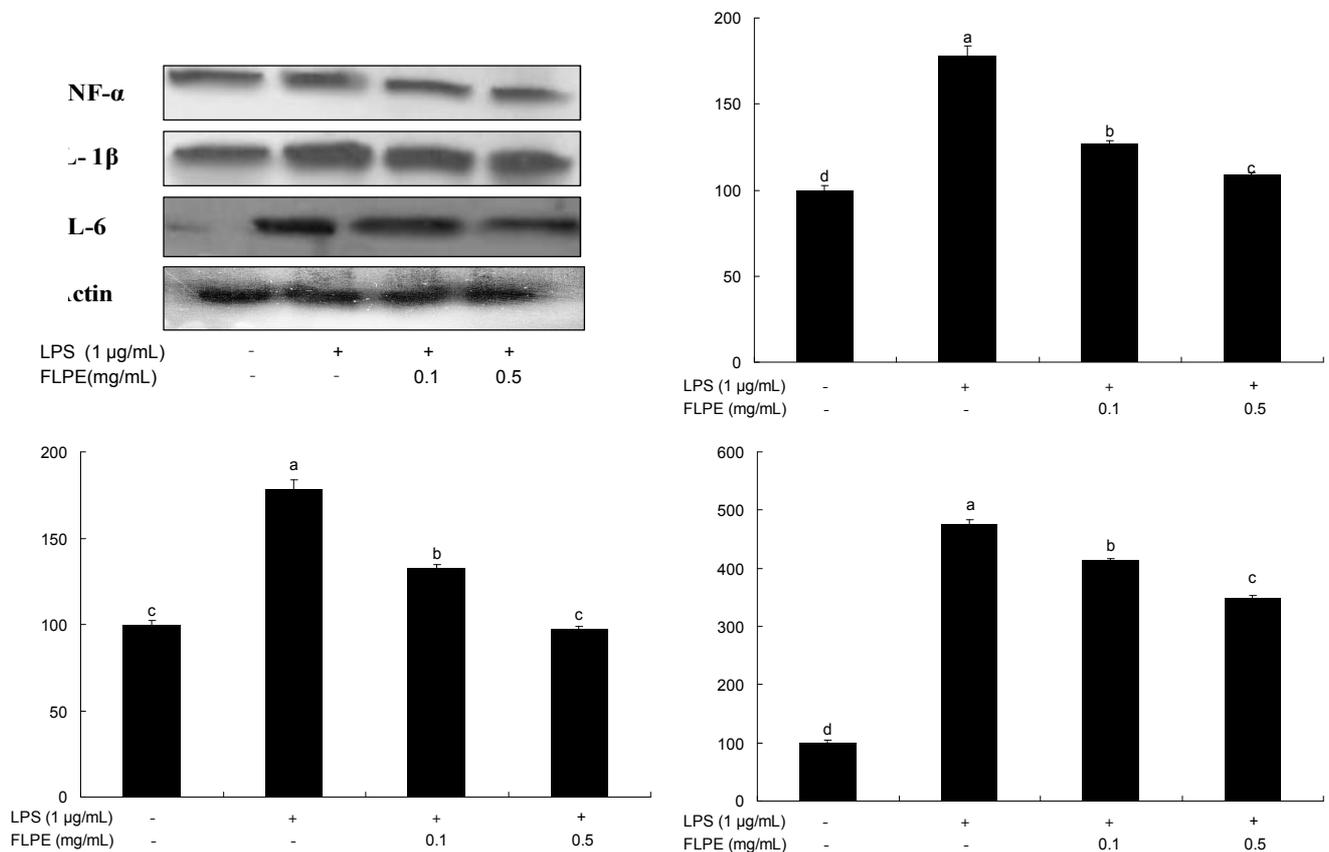
Fig. 4. Inhibitory effects of FLPE on TNF-α, IL-1β and IL-6 production in LPS-stimulated RAW 264.7 cells. Equal amounts of cell lysates (30 μg) were subjected to electrophoresis and analysed for TNF-α, IL-1β and IL-6 activity by Western blot. The cells were first incubated with or without indicated concentrations of FLPE for 2 hr, and then incubated with LPS (1 μg/mL) for 20 hr. Each value is expressed as mean±SD (n=3). [a-d]Values with different alphabets are significantly different at $p < 0.05$ as analyzed by Duncan's multiple range test. FLPE: fermented *Liriope platyphylla* extracts with *Saccharomyces cerevisiae*.

ment with FLPE in a concentration-dependent manner (Fig. 5). NF-κB is an important target for preventing or ameliorating inflammatory response. When macrophages are activated by inflammatory stimuli, NF-κB is known to induce the expressions of pro-inflammatory mediators, such as iNOS and COX-2 (37). To determine the inhibitory effects of FLPE on iNOS and COX-2 expression, Western blotting was used. It is well known that LPS strongly upregulates iNOS and COX-2 levels in RAW 264.7 cells. As shown in Fig. 5, LPS significantly enhanced the expression of iNOS and COX-2. However, these expression levels were reduced markedly ($p < 0.05$) by the treatment of FLPE. iNOS is induced by various inflammatory stimuli, such as LPS or inflammatory cytokines in macrophage. iNOS catalyses the formation of a large amount of NO, which plays a key role in various types of inflammation (38). COX-2 is involved in inflammatory responses by mediating the production of prostaglandins (39). Co-induction or co-regulation of iNOS and COX-2 has been demonstrated in a number of cell culture studies and in animal inflammatory models. Both iNOS and COX-2 are inducible forms of enzymes upregulated in response to inflammation challenge. Expression of iNOS and COX-2 can be regulated by the activation of NF-κB (40,41). The results of this study indicate that NF-κB is inhibited by FLPE, and that it consequently inhibited the expression of iNOS and COX-2.

In conclusion, the present study showed that FLPE decreased intracellular ROS levels and increased antioxidant enzyme activities. Also, FLPE inhibited NO production and the expressions of pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) and inflammatory mediators (iNOS and COX-2) in LPS-stimulated RAW 264.7 cells. Thus, this study shows FLPE could be effective for inhibition of the inflammation process. However, further studies are needed to identify which ingredients in FLPE inhibit the inflammation.

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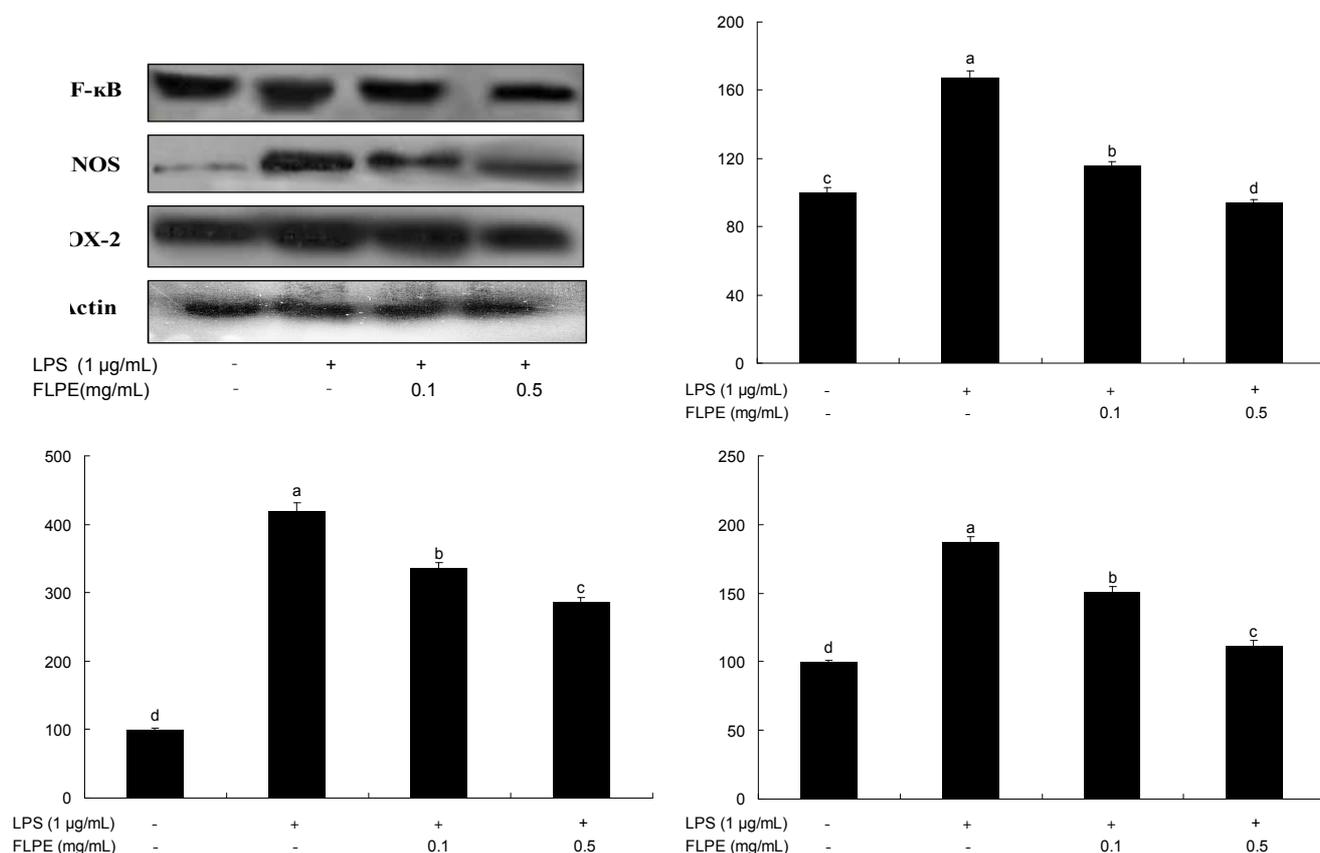
Fig. 5. Inhibitory effects of FLPE on NF-κB, iNOS and COX-2 levels in LPS-stimulated RAW 264.7 cells. Equal amounts of cell lysates (30 μg) were subjected to electrophoresis and analyzed for NF-κB, iNOS and COX-2 activity by Western blot. The cells were first incubated with or without indicated concentrations of FLPE for 2 hr, and then incubated with LPS (1 μg/mL) for 20 hr. Each value is expressed as mean±SD (n=3). [a-d]Values with different alphabets are significantly different at p<0.05 as analyzed by Duncan's multiple range test. FLPE: fermented *Liriope platyphylla* extracts with *Saccharomyces cerevisiae*.

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