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Anti-inflammatory Activity of Extracts from Ultra-Fine Ground *Saururus chinensis* Leaves in Lipopolysaccharide-Stimulated Raw 264.7 Cells

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Abstract Bioactive components of ultra-fine ground *Saururus*, the extraction yield increases when the leaves are ultra-fine ground. Comparison of normal-ground and ultra-fine ground *Saururus chinensis* leaves showed that the solid content and anti-inflammatory activity of ultra-fine ground extracts was higher than that of normal-ground extracts. Lipopolysaccharide (LPS)-stimulated Raw 264.7 cells were treated with different concentrations of *Saururus chinensis* extract and the amount of nitric oxide (NO) was determined; LPS-treated cells produced 2 times more NO than cells that were not treated with LPS. Moreover, the NO production in cells treated with *Saururus chinensis* extract was inhibited in a concentration-dependent manner. Because the stimulant-induced NO production is regulated by the inducible nitric oxide synthase (iNOS), we measured the iNOS protein level to elucidate the mechanism by which the NO production was inhibited. We found that the amount of iNOS decreased dose-dependently. It was reduced by 53% at a *Saururus chinensis* extract concentration of 100 μg/mL. The protein expression of cyclooxygenase-2 (COX-2) in LPS-treated Raw 264.7 cells was inhibited by 31% at 100 μg/mL of *Saururus chinensis* extract. Gel shift of the nuclear factor kappa B-DNA complex occurred in LPS-treated cells and the intensity of the band decreased gradually in a concentration-dependent manner. Ultra-fine ground *Saururus chinensis* extract had a concentration-dependent inhibitory effect on the production of prostaglandin E_2, tumor necrosis factor α, interleukin 1β (IL-1β), IL-6, and IL-8 in LPS-treated Raw 264.7 cells, i.e., at 50 μg/mL of *Saururus chinensis* extract, their levels were decreased by 53, 67, 52, 37, and 21% respectively.

Keywords anti-inflammation · ethanol extracts · *Saururus chinensis* · ultra-fine grinding

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Introduction

Saururus chinensis is an herb that belongs to the Saururaceae family. It is found in South Korea, China, Japan, and other East Asian countries. *Saururus chinensis* is a medicinal herb (Kwon, 1999a; Park and Lee, 2000) used for the treatment of edema, detoxification, diabetes, high blood pressure, hepatitis, and jaundice. Because *Saururus chinensis* is an edible herb, it could also be used as food ingredient such as in health-promoting teas. *Saururus chinensis* leaves contain high amounts of phenolic compounds such as quercetin, quercetrin, isoquercitrin, avicularin, rutin, etc., which have anti-cancer effects. The root is known to be effective for the treatment of suppurative mastitis, urethral pain, lifestyle diseases, and high blood pressure because it contains amino acids, organic acids, saccharides, and hydrolysable tannins (Choe et al., 1994; Choe, 1999; Kwon, 1999b; A society for Korea medicinal botany, 2001; Lee, 2002). The flavonoids quercetin and quercetrin are natural antioxidants that exist in the plant kingdom and have been indicated to prevent aging, cancer, and cardiac diseases by inhibiting fat oxidation, removing active oxygen species, and inhibiting oxidative stress (Kwak, 1988; Kim et al., 2005). The components contained in *Saururus chinensis* have been shown to have protective activity in interstitial cell, exert antibacterial and antioxidant effects, and strengthen capillary vessels (Kwon, 1996; Kim and Song, 2000; Lee et al., 2000; Lee et al., 2001).

Flavonoids, which are natural antioxidants that exist in the plant kingdom, are used in various fields such as functional food, medicines, and functional cosmetics because of they have been shown to prevent cancer and aging. In addition, many recent reports showed that the bioactive substances separated from natural substances were identified as flavonoid compounds (Lim et al., 1996; Park et al., 1998).

Macrophages release and produce cytokines, active oxygen species, and nitric oxide (NO) when activated by small amounts of lipopolysaccharide (LPS) (Oshima and Bartsch, 1994; Gross and Wolin, 1995; Kroncke et al., 1998; Stuehr, 1999; So et al., 2004). Macrophages, which are phagocytes and immune cells produced in the bone marrow, are immunocytes that destroy cancer cells or heterocytes by secreting cytotoxic substances such as hydrogen peroxide or NO (Nirupama et al., 2005). They are a major player in the defense mechanism of the immune system and are involved in inflammatory responses by secreting factors such as cytokines, phosphatases, interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) (Anfernee et al., 2005; Naoko et al., 2005; Syu-ichi et al., 2005; Iwona et al., 2006).

Ultra-fine grinding is an effective technique to increase the solubility of a material that is difficult to dissolve by maximizing its surface. In addition, the extraction yield is increased and phenolic compounds, which are useful substances, are relatively easily obtained. The ultra-fine grinding technique is superior to other primary processing techniques. This technique increases the solubility of existing medicinal herbs (as demonstrated by the increased rate by which they are absorbed in the body), which can be expected to be used in functional foods, and medicinal materials, and high-value material.

The main object of this study was to increase the high value of the selected material by investing the biological activity against inflammation as functional food, medicinal material and extraction yield of solid using an ultra-fine grinding process.

Materials and Methods

Preparation of sample powders by normal grinding and ultra-fine grinding. *Saururus chinensis* used in this experiment were purchased in an oriental herb store at Yakryung market in Daegu city. *Saururus chinensis* was used after normal-grinding to powder (ASTM 40 mesh under size). Ultra-fine grinding *Saururus chinensis* was prepared as ultra-fine ground powder (125 μm ISO mesh size, ASTM 140 mesh: under size) at a grinding speed of 20 kg per hour by ultra-fine grinding machine of 10 L capacity (MKFS10-1, Koen 21 Co. Ansan, Korea).

Extraction process of *Saururus chinensis*. To obtain the extracts, 50% ethanol was added to the sample and the mixture was homogenized with a homogenizer at 20,000 rpm for 1 min. The sampels were then extracted by shaking for 24 h. The extracts were used in the experiment after filtering with Whatman No. 1 filter paper and concentrated in a rotary vacuum evaporator (Eyela NE, Tokyo, Japan) if needed.

Determination of the inhibitory effect on hyaluronidase. The inhibitory effect on hyaluronidase (HAase) was determined by measuring the decrease in the absorbance of glucoxazoline, an *N*-acetylglucosamine derivative. This compound is formed in the presence of HAase by the reaction of sodium hyaluronic acid (HA) (added as substrate) with *ρ*-dimethylaminobenzaldehyde (DMAB), resulting in a color change. Briefly, 0.05 mL HAase solution (7,900 U/mL) dissolved in 0.1 M acetate buffer (pH 3.5) and 0.1 mL of 50% ethanol extracts from *Saururus chinensis* were mixed and incubated for 20 min at 37°C. Then, 12.5 mM $CaCl_2$ (0.1 mL) was added and HA (12 mg/mL) dissolved in 0.1 M acetate buffer (pH 3.5) was added as substrate. The mixture was incubated of 40 min. Next, 0.1 mL of 0.4 N $K_2B_4O_7$ and 0.1 mL of 0.4 N NaOH solution was added to the reacted mixture, incubated for 30 min in a water bath, and then completely cooled. Then, 3 mL DMAB, which is a color forming reagent, was added to the cooled mixture and incubated for 20 min at 37°C. Absorbance was measured at 585 nm and the inhibitory activity was calculated as previously reported (Tolksdorf et al., 1949). The inhibitory activity (%) was calculated as follows: [1−absorbance of sample/absorbance of control]×100.

Cell culture to investigate the anti-inflammatory effect. Raw 264.7 cells, a murine macrophage cell line, was purchased from the Korean Cell Line Research Foundation. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin. In general, cells were seeded onto cell culture dishes at a density of $2–3\times10^6$/mL and cultured in Dulbecco's modified Eagle's medium for 72 h at 37°C and 5% CO_2. For the experiments, cells were grown to 80% confluence and used up to passage 20. In addition, cells were incubated for 12 h without fetal bovine serum before the experiments (Cho and An, 2008).

NO measurement. The NO concentration was determined as nitrite and nitrate in cell supernatants. The Griess reagent (Sigma, USA) was used for measuring because the oxidation of nitrite to nitrate is stabilized. Briefly, 2×10^6 cells were seeded in 6-well plates and cultured to 80% confluence. Then, they were washed twice with phosphate-buffered saline (PBS) and incubated for 12 h in serum-free medium. Next, cells, except the normal group, were stimulated by adding LPS (50 μM). After 2 h, water and ethanol extracts (10 μg/mL and 100 μg/mL) were added to the cells. In the control group, an equal amount of distilled water was added instead of the extracts. The cells were incubated for 24 h. Then, the supernatants were collected, the Griess reagent was added, and the samples were incubated for 10 min in the dark. The amount of NO was determined by measuring the absorbance at 540 nm (Cho and An, 2008). The inhibitory activity (%) was calculated as follows: [1−absorbance of sample/absorbance of control]×100.

Analysis of inducible nitric oxide synthase (iNOS) expression by western blot. After Raw 264.7 cells reached 80% confluence, the culture medium was replaced with serum-free Eagle's minimum essential medium and then subjected to different treatments. After incubation under these conditions, supernatants were removed

after the indicated time periods and the cells were washed twice with PBS. The cells were collected with a scrapper and eluted by adding lysis buffer. The protein concentration in each sample was determined by measuring the optical density. A standard curve for bovine serum albumin was plotted and the protein concentration was calculated from this curve. The obtained protein samples were dyed with Coomassie blue and then used for western blot. Proteins were separated by electrophoresis using a 10% sodium dodecyl sulfate-polyacrylamide gel. After electrophoresis, the gel was immersed in transfer buffer for 10–15 min. The gel was transferred onto a nitrocellulose membrane by electroblotting for 1 h at 190 mA. Successful protein transfer was confirmed by immersing the gel in Ponceau S for 2 min. The membrane was washed twice with PBS and incubated overnight in blocking buffer to reduce background staining. After washing twice, the membrane was incubated first with a primary antibody (iNOS; BD Biosience; 1:100 and glyceraldehyde-3-phosphate dehydrogenase; Santa Cruz; 1:1000) and then with a secondary antibody (1:1,000). The membrane was then washed several times with 0.5% Tween 20 in PBS and specific protein bands were visualized using an ECL kit (Amersham Pharmacia, UK) (Cho and An, 2008).

Analysis of COX-2 expression by western blot. To measure the amount of cyclooxygenase-2 (COX-2) protein expressed in Raw 264.7 cells, cells were seeded at a density of 2×10^4 cells/mL in 100 mm tissue culture dishes and then incubated for 24 h. Then, the medium was removed and cells were incubated with different concentrations of extract. After the indicated times, the medium was removed and the cells were washed twice with PBS. The cell was lysed in 100 μL lysis buffer. The samples were centrifuged at 4°C and 12,000 rpm for 20 min. Only the supernatants were collected and transferred to new tubes. They were stored at −20°C for protein determination or future use. The protein concentration in the supernatants was determined by the Bradford method and 20 μL of each protein sample were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel. The separated proteins were transferred to a polyvinylidene fluoride membrane using a semi-dry transfer cell (Bio Rad, Hercules, CA, USA). The membrane was incubated for 1 h in blocking buffer (5% skim milk in TBST). Then, it was washed thrice with TBST (10 min each) and incubated with primary antibody overnight at 4°C (COX-2; Cayman; 1:1000 and glyceraldehyde-3-phosphate dehydrogenase; Santa Cruz; 1:1000). The membranes were then again washed thrice with TBST (10 min each) and incubated with secondary antibody for 2 h at room temperature (horseradish peroxidase-conjugated mouse anti-rabbit IgG and bovine anti-goat IgG; Santa Cruz; 1:1000). After washing thrice, the membrane was incubated with ECL solution (Millipore, bedford, MA, USA) in the dark. The membrane was exposed to X-ray film and the intensity of each band was measured using a Molecular Imager (Bio-Rad) (Cho and An, 2008).

Measurement of cytokine production. Macrophages were seeded on 6-well plates at a density of 1×10^6/mL and treated with different concentrations of LPS for different times. After treatment with LPS (1 μg/mL) for 1 h, cells were incubated in medium for 24 h. Then, the medium was collected and stored at −70°C. The amount of TNF-α, IL-1β, IL-6, IL-8, and prostaglandin E_2 (PGE_2) in the medium was determined using enzyme immunoassay kits. The amount of each cytokine was calculated using standard curves derived from the corresponding reference standards (Byun et al., 2005; Iwona et al., 2006).

Table 1 Inflammatory activity in ethanol extracts from *Saururus chinensis* by ultra-fine grinding technique

Grinding technique	Solid content (mg/g)	Hyaluronidase inhibition (%)
Normal grinding	174.4±2.5	71.6±2.3
Ultrafine grinding	426.1±3.2*	91.5±2.1*

The data were expressed as the mean±standard deviation (n=6), p <0.05.

Result and Discussions

Determination of the solid content and anti-inflammatory activity in extracts obtained using a grinding technique. We compared the extraction yield and anti-inflammatory activity of the solids. As shown in Table 1, the extraction yield and anti-inflammatory activity were increased when ultra-fine ground powder was used. Cho et al. (2008) reported that when red ginseng was ground through a 10–40 mesh, the extraction yield was 1.56 times higher compared to raw ginseng, showing that the extraction yield increases as the particle size decreases. Thus, extracts from ultra-fine ground *Saururus chinensis* leaves were used for the anti-inflammatory studies in Raw 264.7 cells. As shown in Table 1, the anti-inflammatory activity of the ultra-fine ground sample was higher than that of normal-ground sample in ethanol extracts of *Saururus chinensis*. With regard to the effect of ultra-fine grinding on the extraction yield, more bioactive substances were eluted from ultra-fine ground samples. Comparison of the different types of grinding methods revealed that ultra-fine grinding resulted in a 27.8% higher HAase inhibitory activity compared to normal-grinding. This result showed that the anti-inflammatory activity of *Saururus chinensis* was higher than that of ultra-fine ground persimmon used for the atopy experiment reported by Heo et al. (2010).

Inhibition of NO production in Raw 264.7 cells by treatment with ultra-fine ground *Saururus chinensis* extracts. The endotoxin LPS, known as cell wall constituent of gram-negative bacteria, is also known as a pyrogen. Macrophages are activated by very small amounts of LPS thereby initiating a physiological response by producing and releasing cytokines, arachidonic acid metabolites, active oxygen species, and NO. NO is produced from L-arginine by nitric oxide synthase (NOS). NOS can be classified into endothelial NOS (eNOS), which produces NO for maintaining homeostasis, inducible NOS (iNOS), which is induced by inflammatory factors, and neuronal NOS (nNOS) (Kim et al., 2006). During the inflammatory process in the human body, large amounts of NO, PGE_2, and other inflammatory factors are

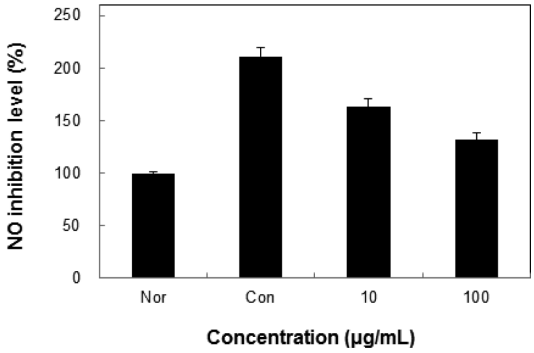

Fig. 1 Effects of extracts from *Saururus chinensis* on the production of NO in Raw 264.7 cells. Raw 264.7 cells were treated with 10 or 100 μg/mL of extracts from *Saururus chinensis* (dissolved in distilled water) for 1 h prior to addition of LPS (10 ng/mL). The cells were further incubated with LPS for 24 h. Control cells were incubated with vehicle alone. The concentrations of nitrite and nitrate in the culture medium were monitored as described in the experimental procedures. Data represent the mean ± SD of nine separate experiments. LPS: 10 ng/mL LPS treatment.

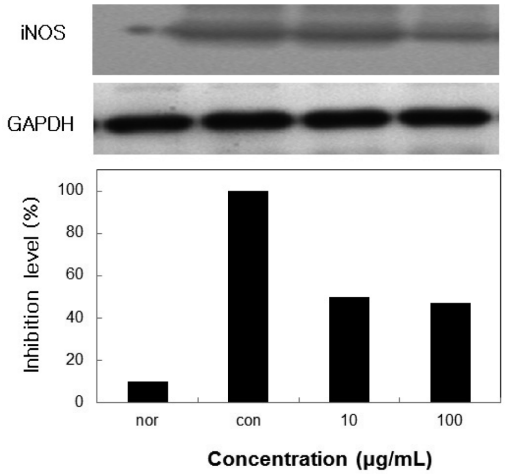

Fig. 2 Effects of extracts from ultra-fine ground *Saururus chinensis* on the expression of iNOS protein in LPS-stimulated Raw 264.7 cells. Raw 264.7 cells were treated with 10 and 100 μg/mL of extracts from *Saururus chinensis* (dissolved in distilled water) for 1 h prior to addition of LPS (10 ng/mL). The cells were further incubated with LPS for 24 h. Control cells were incubated with vehicle alone. The concentrations of nitrite and nitrate in the culture medium were monitored as described in the experimental procedures. Data represent the mean ± SD of nine separate experiments. The histogram shows the densitometric quantification of iNOS protein normalized to glyceraldehyde-3-phosphate dehydrogenase. LPS: 10 ng/mL treatment.

produced by iNOS and COX-2. NO plays a role in various physiological processes such as host defense, signal transfer, neurotoxicity, angiectasis, etc. NOS, which forms NO, can be classified into 3 different isoenzymes, types I, II, and III, according to the physicochemical aspect. Type I (nNOS) and type II (eNOS) are classified as composing NOS (constitutive cNOS) because they exist inside the cell. Type III is classified as induction-type NOS (iNOS) because it is only expressed inside the cell following exposure to certain stimulants such as LPS, cytokines, or bacterial toxins. These NOS species produce NO when transforming L-arginine to L-citrulline. In general, NO production has important roles such as destroying bacteria or removing tumors; however, excessive NO production causes inflammation, organ damage, and genetic modifications. The amount of NO was measured in order to evaluate the effect of *Saururus chinensis* extracts on the NO production in Raw 264.7 treated with different concentrations of the extract (Fig. 1). Cells treated with LPS produced 2 times more NO than cells not treated with LPS. The NO expression was inhibited in a concentration-dependent manner. The NO production in cells treated with *Saururus chinensis* extract at 100 μg/mL was inhibited by ≥70% compared to normal control cells.

Inhibition of iNOS protein expression in LPS-stimulated Raw 264.7 cells by treatment with ultra-fine ground *Saururus chinensis* extracts. NO is a free radical that is produced by various cells throughout the body where it has various biological functions such as vasodilation, smooth muscle contraction, neural signaling transfer, platelet aggregation inhibition, immunity control, etc. NO mediates not only anti-cancer and antimicrobial effects but also causes inflammatory diseases. However, excessive NO production will cause an accelerated inflammatory response, septic shock characterized by excessive vasodilation, inhibition of wound healing, *Lupus erythematosus*, decreased immunity decrease, and apoptosis. The production of NO is regulated by NOS. Among the NOS species, iNOS, which is induced by stimulation, produces large amounts of NO over an extended period of time. The produced NO activates guanylyl cyclase and induces cytotoxicity in surrounding organs. A decrease in the protein level of iNOS in LPS-stimulated Raw 264.7 cells is thought to indicate an anti-inflammatory effect. iNOS defends the host from contagiousness pathogens, including viruses. In addition, iNOS known to be closely involved in various inflammatory diseases, circulatory disorders, and cancer. To elucidate the relationship of inhibited NO production and iNOS protein, we used western blot analysis and determined the amount of iNOS protein in the cytoplasm. The results are shown in Fig. 2. In cells treated with 100 μg/mL of *Saururus chinensis* extract, the amount of iNOS was decreased by 53%. This result shows that the *Saururus chinensis* extract has a higher iNOS inhibitory activity than the salidroside extract (30% inhibitory activity) used by Won *et al.* (2008). Thus, *Saururus chinensis* extract is a material with anti-inflammatory activity as demonstrated by its ability to inhibit the LPS-induced expression of iNOS protein.

Inhibition of COX-2 protein expression in LPS-stimulated RAW 264.7 cells by treatment with ultra-fine ground *Saururus chinensis* extracts. COX-2 is produced by pro-oxidant and pro-inflammatory stimuli that activate mitogen-activated protein kinase-1 and nuclear factor kappa B (NF-κB). COX-2 is pivotal in inducing an inflammatory response by increasing the prostaglandin synthesis. The expression of COX-2 in monocytes increases the levels of IL-1β, TNF-α, phosphatidic acid, fibroblast growth factor, etc., which are pro-inflammatory agents. The

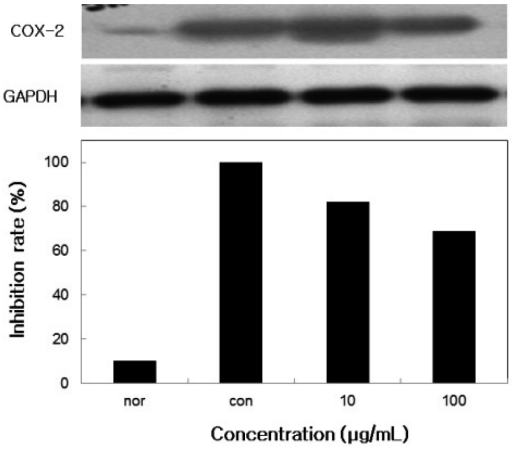

Fig. 3 Effects of extracts from ultra-fine ground *Saururus chinensis* on the expression of COX-2 protein in LPS-stimulated Raw 264.7 cells. Raw 264.7 cells were treated with 10 and 100 μg/mL concentrations of extracts from *Saururus chinensis* dissolved in distilled water for 1 h prior to addition of LPS (10 ng/mL). The cells were further incubated with LPS for 24 h. Control cells were incubated with vehicle alone. The concentrations of nitrite and nitrate in the culture medium were monitored as described in the experimental procedures. Data represent the mean ± SD of nine separate experiments. The histogram shows the densitometric quantification of COX-2 protein normalized to glyceraldehyde-3-phosphate dehydrogenase. LPS: 10 ng/mL LPS treatment.

expression of COX-2 can be inhibited by glucocorticoids, IL-4, and IL-1,. The development of a selective inhibitor of COX-2 is a promising approach for the treatment of inflammatory diseases. The anti-inflammatory effect was tested by measuring the COX-2 protein level in macrophages. The amount of COX-2 expressed when cells were treated with LPS is shown in Fig. 3. When the cells were treated with 100 μg/mL of *Saururus chinensis* extract, the amount of COX-2 protein was decreased by 31%. This result showed that the *Saururus chinensis* extract has a higher inhibitory activity than Kaempferol-3-*O*-β-D-sophoroside used by Park et al. (2008) and a similar inhibitory activity as genistein demonstrated by Park et al. (2007). By using western blot analysis, we demonstrated the anti-inflammatory effect of *Saururus chinensis* extracts, i.e., LPS-induced COX-2 expression was inhibited.

Effect of extracts from ultra-fine ground *Saururus chinensis* leaves on NF-κB transcription in LPS-stimulated Raw 264.7 cells. NF-κB is a transcription factor involved in inflammatory responses, immune reactions, and the expression of various genes. It is a pivotal factor in tumorigenesis, autoimmune diseases, and inflammatory diseases. NF-κB is known to be in the inactive state in the cytoplasm, but becomes activated when stimulated by inflammatory factors such as LPS. This stimulation causes the degradation of IκB and release of NF-κB, which translocates to the nucleus where it induces the expression of cytokines, iNOS, COX-2, and intercellular adhesion molecules An inflammatory response is induced by vascular cell adhesion molecules. In this study, the binding of nuclear NF-κB to DNA was investigated. Gel shift occurred in LPS-treated cells and the intensity of this band decreased gradually and in a concentration-dependent manner

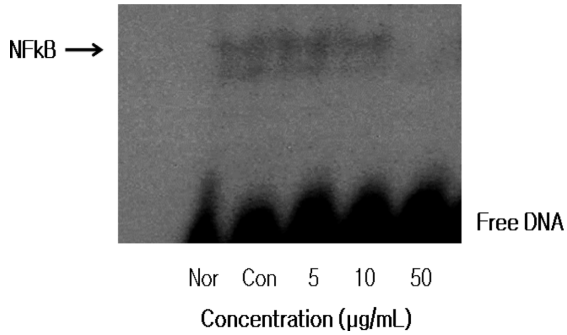

Fig. 4 Effect of ultra-fine ground *Saururus chinensis* extracts on amount of nuclear NF-κB. Raw 264.7 cells were incubated with various concentrations (5, 10, and 50 μg/mL) of solvent fractions from *Saururus chinensis* extracts for 1 h and then treated with 1 μg/mL of LPS for 24 h.

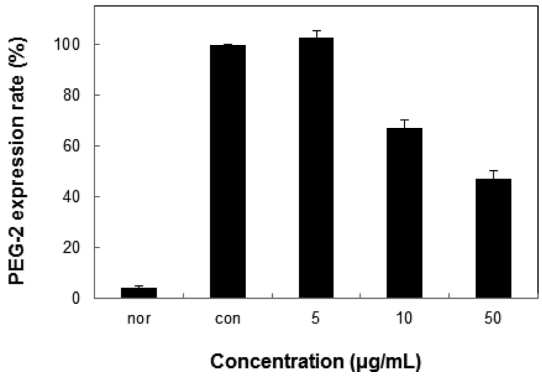

Fig. 5 Inhibition rate of ultra-fine ground *Saururus chinensis* extracts on the expression of PGE_2. Raw 264.7 cells were incubated with various concentrations (5, 10, and 50 μg/mL) of *Saururus chinensis* extracts for 1 h and then treated with 1 μg/mL of LPS for 24 h.

by treatment with *Saururus chinensis* extracts (Fig. 4). This result was similar to the observations made by Kim et al. (2008). They observed a super shift and the intensity of the band decreased as the concentration of isoliquiritigenin increased (0.4–1.6 μM). Similar result were reported by Liu et al. (2009) who observed a decrease in the intensity of the super shift band as the concentration of 1,6-diacetylbritannilactones increased.

Inhibitory effect of extracts from ultra-fine ground *Saururus chinensis* leaves on PGE_2 expression in LPS-stimulated Raw 264.7 cells. Prostaglandins are substances that are locally activated and synthesized from arachidonic acid by COX. PGE_2 is an important factor that induces inflammation responses causing erythema, edema, and pain. We measured the amount of PGE_2 produced by LPS-stimulated Raw 264.7 cells that were treated with *Saururus chinensis* extract. As shown in Fig. 5, the amount of PGE_2 decreased in a dose-depend manner, it was decreased to 53% compared to control cells at a concentration of 50 μg/mL *Saururus chinensis* extract. Yoon et al. (2007) reported that the production of PGE_2 was inhibited when cells were treated with 50 μg/mL mugwort extract. These results show that *Saururus chinensis* extracts could be effective for treating inflammatory diseases.

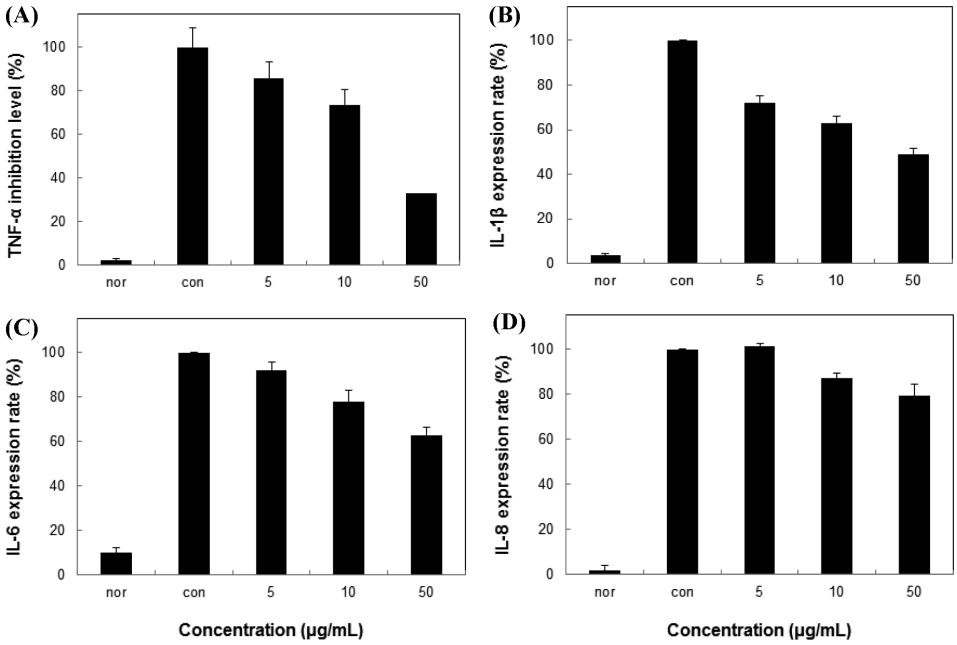

Fig. 6 Inhibition rate of ultra-fine ground *Saururus chinensis* extracts on cytokine expression. Raw 264.7 cells were incubated with various concentrations (5, 10, and 50 μg/mL) of *Saururus chinensis* extract for 1 h and then treated with 1 μg/mL of LPS for 24 h. A: TNF-α, B: IL-1β, C: IL-6, D: IL-8.

Inhibitory effect of extracts from ultra-fine ground *Saururus chinensis* leaves on the cytokine production in LPS-stimulated Raw 264.7 cells. To determine if *Saururus chinensis* extracts inhibit the production of pro-inflammatory cytokines in LPS-stimulated Raw 264.7 cells, the amounts of TNF-α, IL-1β, IL-6, and IL-8 were determined. As shown in Fig. 6, the production of TNF-α, IL-1β, IL-6, and IL-8 was decreased by *Saururus chinensis* extracts in a concentration-dependent manner. At a concentration of 50 μg/mL *Saururus chinensis* extract, the amount of TNF-α, IL-1β, IL-6, and IL-8 was decreased by 67, 52, 37, and 21%, respectively, compared to control cells. Yoon *et al.* (2007) reported that mugwort extract at a concentration of 50 μg/mL inhibited the LPS-induced production of cytokines such as TNF-α, IL-1β, and IL-6 in Raw 264.7 cells. Cheon et al. (2009) reported that the LPS-induced production of TNF-α, IL-1β, and IL-6 in Raw 264.7 cells was inhibited by treatment with a *Bulnesia sarmienti* hot water extract at a concentration of 200 μg/mL.

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