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Anti-inflammatory Activity of *Sorghum bicolor* (L.) Moench var. Hwanggeumchal Grains in Lipopolysaccharide-stimulated RAW264.7 Murine Macrophage Cell Line

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To investigate the anti-inflammatory activity of the grains of sorghum, three *Sorghum bicolor* (L.) Moench variants (Hwanggeumchal, Huinchal, and Chal) being cultivated in Korea, the 80% ethanol (EtOH) extracts of individual sorghum grains were compared for their inhibitory activity against nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated RAW264.7 murine macrophage cell line. Among them, the EtOH extract of sorghum Hwanggeumchal grains could exert the highest inhibitory effect on the LPS-induced NO production. However, under these conditions, the viability of RAW264.7 cells was not affected. When the EtOH extract of sorghum Hwanggeumchal grains was sequentially fractionated with n-hexane, methylene chloride (MC), ethyl acetate (EtOAc), and n-butanol, the anti-NO production activity was predominantly detected in both MC and EtOAc fractions. In particular, treatment with the MC fraction reduced dose-dependently the expression levels of iNOS, COX-2 and pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in LPS-stimulated RAW264.7 cells. Simultaneously, the MC fraction could prevent LPS-induced activating phosphorylation of p38 mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinase (JNK) and extracellular signal-regulated kinase (ERK). HPLC analysis of the MC fraction showed gentisic acid and naringenin as the major phenolic components. Both gentisic acid and naringenin commonly exhibited a potent inhibitory activity against LPS-induced NO production in RAW264.7 cells. Together, these results provide the evidence of the inhibitory activity of Hwanggeumchal grains on LPS-induce inflammatory responses in RAW264.7 murine macrophage cells and also suggest that sorghum grains possess beneficial health effects which can be applicable in development of the grain-based functional foods.

Key words : Anti-inflammation, anti-NO production activity, beneficial health effects, RAW264.7 cells, sorghum Hwanggeumchal grains

Introduction

Inflammation is a process of wound healing and restoration of tissue function by eliminating the injurious stimuli in organisms [15]. However, over-production of pro-inflammatory cytokines, prostaglandins (PGs) and free radicals such as reactive oxygen radicals (ROS) and reactive nitrogen species (RNS) by the inflammation response can cause other cells to develop inflammation-mediated diseases [5, 22]. Moreover, it has been reported that chronic inflammation increases with aging and contributes to onset of age-related diseases in aging organisms [2, 30]. These findings suggest that effective control of inflammation is very important in the prevention of chronic inflammation-related diseases including arthritis, chronic hepatitis, type II diabetes, cardiovascular disease, and various cancers.

The lipopolysaccharide (LPS)-induced response of macrophages plays an important role in the inflammation-related diseases. The LPS-induced inflammatory diseases were reported to correlate with overproduction of pro-inflammatory mediators, such as nitric oxide (NO), prostaglandin E_2 (PGE_2), and interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α), by activated macrophages [20, 33]. Nitric oxide (NO) as an important pro-inflammatory mediator is formed by the inducible NO synthase (iNOS)-catalyzed oxidation of L-arginine during early stage of inflammation response of macrophages [11, 24]. The expression of these

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pro-inflammatory mediators as well as iNOS in LPS-stimulated macrophages is known to be upregulated at the transcriptional level by a key transcriptional factor NF-kB, of which activation is dictated by LPS-stimulated Toll-like receptor 4 (TLR4) signaling pathway [10].

As an attempt to improve inflammation-related health problems using natural materials, many crops, medicinal and wild edible plants were evaluated for their anti-inflammatory and anti-oxidative properties and potential of anti-inflammatory functional foods [32]. In the literature, miscellaneous cereal grains are among the more promising sources that can be applicable as functional foods effective in ameliorating metabolic syndromes [7, 8, 16]. As an anti-inflammatory phytochemical component in Njavara rice, a flavonolignan compound, tricin 4′-O-(threo-β-guaiacylglyceryl) ether appears to down-regulate the expression levels of iNOS and cyclooxygenase-1 (COX-2) in LPS-stimulated RAW264.7 cells [18]. In addition, aleurone layer extracts of wheat, oats, rice, corn, and barley can exert anti-inflammatory activity by reducing TNF-α level in LPS-treated U937 human pro-monocytic myeloid leukemia cell line [1]. Previously, we have observed that 80% ethanol extract of hwanggeumchal sorghum (*Sorghum bicolor* L. Moench var. Hwanggeumchal), glutinous sorghum (*Sorghum bicolor* L. Moench var. Chal), and barnyard millet (*Echinochloa crus-galli var.* Frumentacea) grains possess efficient antioxidant activities which can protect human HL-60 cells from oxidative stress-mediated cytotoxicity [27]. In addition, we have reported that kaempferol and bichanin A are major ingredients possessing anti-inflammatory activity in barnyard millet grains [21].

Sorghum is a major crop in arid and semi-arid tropics and one of the five major crops of the world, along with rice, barley, wheat, and maize. The nutritive and biological studies on the grains of have demonstrated that they are rich in various bioactive phytochemicals including tannins, phenolic acids, anthocyanins, phytosterols, and policosanols. Several studies have reported that the extracts of sorghum grains possess anti-oxidant activity [4, 6], anti-carcinogenic and anti-tumor effect [12, 35, 36], anti-thrombotic and fibrinolytic effects [25], antimicrobial activity [19], and anti-inflammatory activity [26]. In relation to the anti-inflammatory activity of *Sorghum bicolor* (L.) Moench var. Hwanggeumchal grains, two purified components (benzoic and cinnamic acid derivatives) have been shown to inhibit LPS-induced NO production in RAW264.7 cells [25]. However, little is known about the biochemical and molecular mechanism responsible for the anti-inflammatory activity of the grains of sorghum.

In this study, we show that gentisic and naringenin-enriched methylene chloride (MC) fraction obtained from sorghum Hwanggeumchal grains exerts a potent anti-inflammatory activity via down-regulation of the expression levels of iNOS, COX-2, and pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) as well as via inhibition of the activating phosphorylation of MAP kinases (MAPKs) such as p38 MAPK, JNK and ERK. These results support that sorghum grains possess beneficial health effects which can be applicable in development of the grain-based functional foods.

Materials and Methods

Reagents, chemicals, antibodies and culture medium

The ECL Western blotting kit was purchased from Amersham (Arlington Height, IL, USA), and Immobilon-P membrane was obtained from Millipore Corporation (Bedford, MA, USA). Horse radish peroxide (HRP)-conjugated anti-mouse IgG and anti-rabbit IgG were obtained from Cell Signaling Technology (Beverly, MA, USA), and HRP-conjugated anti-goat IgG was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The RAW264.7 murine macrophage cell line was purchased from ATCC (Manassas, VA, USA) and was cultured in Dulbecco's modified Eagle's medium (DMEM) which was supplemented with 10% fetal bovine serum (FBS) and 100 μg/ml gentamycin at 37℃ in a humidified 5% CO_2 atmosphere. For the experiments, the cells were grown to 80~90% confluences, and were subjected to no more than 15 cell passages.

Grain preparation and extraction

The grains of three varieties of sorghum (*Sorghum bicolor* (L.) Moench var. Hwanggeumchal, Sorghum bicolor (L.) Moench var. Huinchal, and Sorghum bicolor (L.) Moench var. Chal) were provided by National Institute of Crop Science of Miryang, Korea, in February 2011 and a voucher specimen has been deposited in Laboratory of Immunology, Natural Sciences, Kyungpook National University, Korea. The grinded sorghum grains (100 g) were extracted with 80% ethanol (EtOH) at 80℃ for 3 hr under reflux condensation. The total crude EtOH extract was concentrated under reduced pressure to yield the EtOH extract and then crude extract was suspended in water (1:1). Afterwards, it was successively partitioned with *n*-hexane, methylene chloride (MC), ethyl acetate (EtOAc), *n*-butanol (n-BuOH), yielding a *n*-hexane fraction, MC fraction, EtOAc fraction, n-BuOH fraction and insoluble

water fraction. Each fraction was evaporated to remove organic solvent and then lyophilized until dry and weighted. The yields of *n*-hexane fraction, MC fraction, EtOAc fraction, n-BuOH fraction and insoluble water fraction were 0.579 g, 0.268 g, 0.132 g, 0.541 g, and 0.579 g, respectively, of the total crude extract dry weight.

Cell viability assay

Cytotoxic effect of sorghum extracts on RAW264.7 cells was analyzed using the cell viability assay kit (CellTiter 96® Aqueous One Solution Cell Proliferation Assay) containing 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) reagent. Briefly, RAW 264.7 cells (0.5×10^5/well) were cultured with serial dilutions of a sorghum extract in 96-well plate. At 48 hr after incubation, the medium was removed and replaced with 100 µl fresh culture media and 10 µl MTS solution (Promega, Madison, WI, USA). After incubation for an additional 2 hr, 25 µl 10% sodium dodecyl sulfate (SDS) was added as a stop solution. The absorbance was measured at 490 nm by a plate reader to determine the formazan concentration, which reflects the cell viability.

Nitric oxide assay

As a parameter of NO production, the concentration of nitrite, a stable metabolite of NO, in the culture medium was assessed by Griess reagent [14, 34]. Briefly, RAW264.7 cells (2×10^5 cells/well) were cultured overnight in 96-well plates, and then treated with LPS (0.1 µg/ml) in the absence or in the presence of various concentrations of extracts for 16 hr. The culture supernatant (100 µl) was mixed with an equal volume of Griess reagent for 15 min at room temperature in dark condition, and then the absorbance of the chromophoric azo-derivative molecule was measured using a microplate reader at 540 nm (Molecular Devices, Thermo Max, USA). To ensure the validity of the results, experiments were done in three independent experiments with three replicates per each independent experiment.

Total RNA isolation and RT-PCR

Cells were washed twice in PBS, then total RNA was isolated using the Trizol reagent from Invitrogen (Carlsbad, CA, USA) according to the manufacturer's instructions and DNase I treatment. After RNA quantification by GE NanoVue Spectrophotometer (GE healthcare, Buckinghamshire, UK), 1 µg RNA was reversely transcribed using First strand cDNA synthesis kit (Thermo Scientific, Logan, UT, USA) for cDNA synthesis. Gene expression values were normalized to housekeeping GAPDH gene. GAPDH was amplified with forward (5'-ATCCTGCGTCTGGACCTGGCT-3') and reverse (5'-CT GATCCACATCTGCTGGAAG-3') primers. PCR amplification was done using AccuPower™ PCR PreMix (Bioneer, Seoul, Korea) and specific primers. The following primers were used for PCR: iNOS-forward, 5'-ATGTCCGAAGCA AA-CATCAC-3'; iNOS-reverse, 5'-TAATGTCCAGGAAG TAGGTG-3'; COX-2-forward, 5-CAGCAAATCCTTGCTG TTCC-3; COX-2-reverse, 5'-TGGGCAAAGAATGCAAAC ATC-3'; TNF-α-forward, 5'-TACTGAACTTCGGGG-TGAT CGGTCC-3'; TNF-α-reverse, 5'-CAGCCTTGTCCCTTGAA GAGAACC-3'; IL-6-forward, 5'-GAAATGATGGATGCTT CCAAACTGG-3'; Heme oxygenase-1 (HO-1)-forward, 5'-CAATGTGGCCTTCTCTCTGT-3'; HO-1-reverse, 5'-TTTTG GTGAGGGAACTGTGT-3'. To ensure that the same amount of RNA was being used, the concentration of the total RNA for each sample was confirmed by spectrophotometry and normalized with GAPDH as the message of a housekeeping gene. The PCR products were electrophoresed using 1.2% agarose gel and visualized under UV light after ethidium bromide staining.

Preparation of cell lysate and Western blot analysis

Cellular lysates were prepared by suspending cells (5×10^6) in 300 µl of lysis buffer (137 mM NaCl, 15 mM EGTA, 1 mM sodium orthovanadate, 15 mM $MgCl_2$, 25 mM MOPS, 1 mM PMSF, and 2.5 g/ml proteinase inhibitor E-64, 0.1% Triton X-100, pH 7.2) as described elsewhere [34]. The cells were disrupted by sonication and extracted at 4℃ for 30 min. An equivalent amount of protein lysate (25 µg) was electrophoresed on 4~12% NuPAGE gradient gel (Invitrogen/ Novex, Carlsbad, CA, USA) with MOPS buffer and then electrotransferred to Immobilon-P membranes. Detection of each protein was performed utilizing the ECL Western blotting kit following the manufacturer's instructions.

Statistical analysis

Unless indicated otherwise, each result in this paper is representative of at least three separate experiments. Values represent the mean standard deviation (STD) of these experiments. The statistical significance was calculated with Student's t-test. p values less than 0.05 were considered significant.

Results and Discussion

Comparison of anti-NO production activities of the ethanol extracts of the grains of three sorghum variants in LPS-stimulated RAW264.7 murine macrophage cell line

To compare the anti-inflammatory activities of the grains of three different varieties of sorghum (*Sorghum bicolor* (L.) Moench var. Hwanggeumchal, *Sorghum bicolor* (L.) Moench var. Huinchal and *Sorghum bicolor* (L.) Moench var. Chal), the EtOH extracts (25~200 μl) of the individual grains were tested for their anti-NO production activity using LPS-stimulated RAW264.7 cells.

As shown in Fig. 1A, while the EtOH extracts of three varieties of sorghum grains commonly showed dose-dependent inhibition against LPS-induced NO production in RAW 264.7 cells, the Hwanggeumchal grain extract showed more potent anti-NO production activity than the other sorghum grain extracts and its presence at a concentration of 100 or 200 μg/ml resulted in inhibition of approximately 50% or 90% of the LPS-induced NO production, respectively. Under these conditions, RAW264.7 cell viability was not affected by the EtOH treatment at experimental concentrations tested (Fig. 1B).

These results indicated that the EtOH extract of Hwanggeumchal grains among those of three sorghum variant grains possesses the highest anti-NO production activity, representing the highest anti-inflammatory activity.

Effects of *Sorghum bicolor* (L.) Moench var. Hwanggeumchal grain extracts on viability and production of NO in LPS-stimulated RAW264.7 cells

To further examine the anti-NO production activity of the EtOH extract of sorghum Hwanggeumchal grains, the EtOH extract was sequentially fractionated with n-hexane, MC, EtOAc, and BuOH, and then individual solvent fractions at concentrations ranging from 25-100 μg/ml were investigated for their anti-NO production activities. As shown in Fig. 2A, both MC and EtOAc fractions appeared to inhibit the LPS-induced NO production in RAW264.7 cells more potently compared to the other solvent fractions. In particular, the MC fraction of Hwanggeumchal grains at concentrations of 25, 50, and 100 μg/ml was able to abrogate ~12%, ~48%, and ~95% of the NO production in LPS-stimulated RAW264.7 cells, respectively, with the IC_{50} value of 50.3 μg/ml. However, under these conditions, there was no detectable cytotoxic effect of the MC extract, excluding an involvement of cytotoxic effect, if any, of the MC fraction in the anti-NO production activity observed in LPS-stimulated RAW264.7 cells (Fig. 2B).

Consequently, these results indicated that the MC fraction of sorghum Hwanggeumchal grains at concentrations ranging

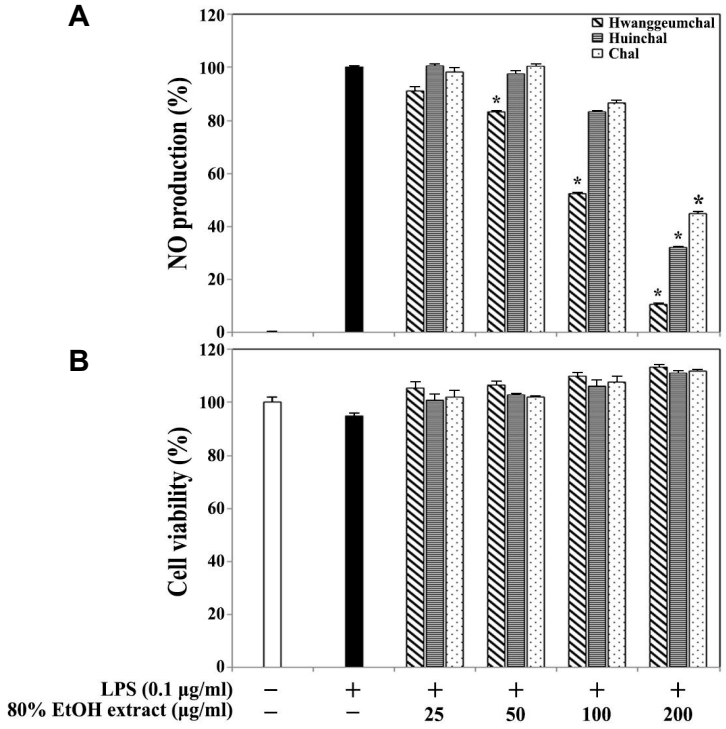

Fig. 1. Effect of 80% EtOH extracts of three different *Sorghum bicolor* (L.) Moench var. Hwanggeumchal, Huinchal, and Chal on the NO production in LPS-induced RAW264.7 (A) and cell viability in RAW264.7 (B). Nitrite was measured using Griess reaction at 20 hr after treatment of LPS (0.1 μg/ml) in the presence or absence of various concentrations (25 μg/ml to 200 μg/ml) of extracts of different grains. The cell viability was determined by the MTS colorimetric assay as described in Materials and Methods. Each value is expressed as mean ± SD (n=3 with six replicates per independent experiment). $p<0.05$ compared with control.

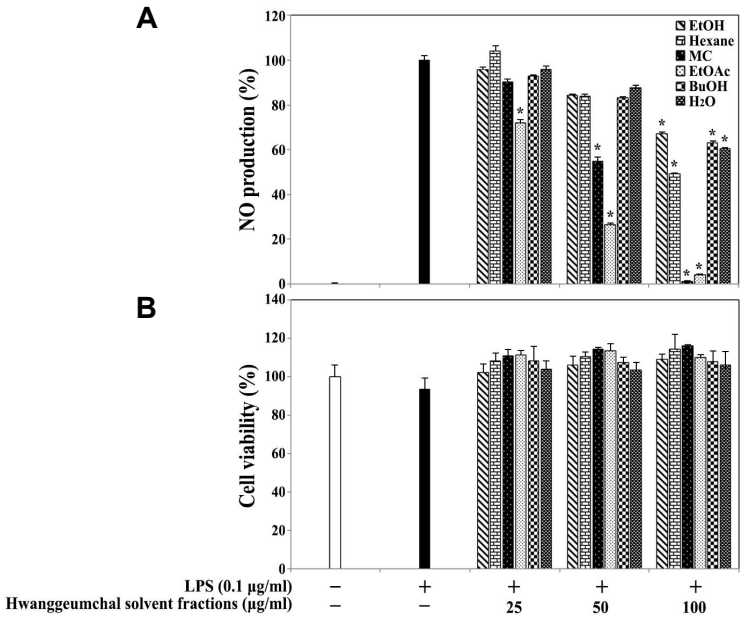

Fig. 2. Effect of *Sorghum bicolor* (L.) Moench var. Hwanggeumchal organic extracts on NO production (A) and cell viability in LPS- induced RAW264.7 cells. Nitrite was measured using Griess reaction at 20 hr after treatment of LPS (0.1 μg/ml) in the presence or absence of various concentrations (25 μg/ml to 100 μg/ml) extracts of *Sorghum bicolor* (L.) Moench var. Hwanggeumchal. The cell viability was determined by the MTS colorimetric assay. Each value is expressed as mean ± SD (n=3 with six replicates per independent experiment). $p<0.05$ compared to control.

from 25 to 100 μg/ml could inhibit dose-dependently the LPS-induced NO production in RAW264.7 murine macrophage cells with the IC_{50} value of 50.3 μg/ml.

Inhibitory effect of the MC fraction from Hwanggeumchal grains on LPS-induced expression of iNOS and COX-2 in RAW264.7 cells

In relation to LPS-induced inflammatory response of macrophages, the production of critical pro-inflammatory mediators such as NO and prostaglandin E2 (PGE2) is known to be governed by the enzymes iNOS and COX-2 [10, 20, 33]. To elucidate the mechanisms underlying the anti-NO production activity of the MC fraction from Hwanggeumchal grains, we sought to examine whether the MC fraction of Hwanggeumchal grains could suppress the expression levels of iNOS and COX-2 in LPS-stimulated RAW264.7 cells by employing the methods of RT-PCR and Western blot analyses.

As a result, the MC fraction (25~100 μg/ml) appeared to reduce dose-dependently the expression of iNOS and COX-2 at mRNA as well as protein levels in LPS-stimulated RAW 264.7 cells (Fig. 3A and 3B). At the same time, the presence of MC fraction was able to decrease the levels of mRNA expression of pro-inflammatory cytokines such as IL-1β, IL-6 and TNF-α in LPS-stimulated RAW264.7 cells (Fig. 3C).

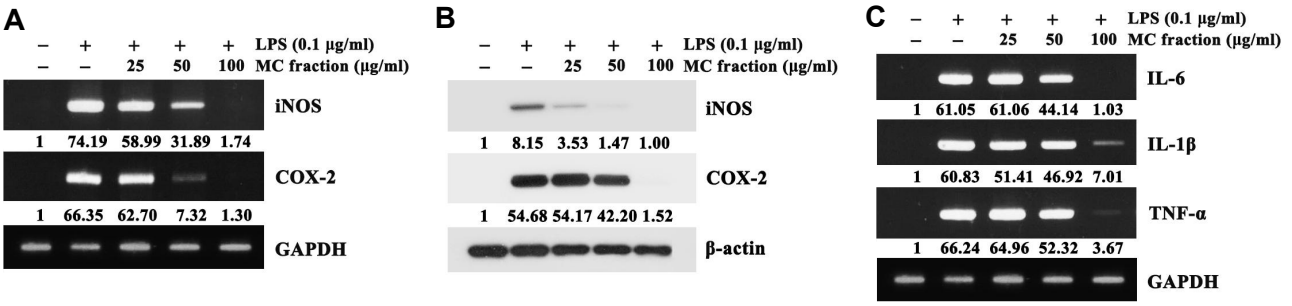

Fig. 3. Effect of the MC fraction on LPS-induced iNOS, COX-2 and pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) expression in RAW264.7. Cells were pretreated with or without the MC fraction at indicated concentrations and LPS (0.1 μg/ml) for 20 hr. (A and C) Gene expression of RT-PCR bands detected in RAW264.7 and LPS-induced RAW264.7 cells. (B) Western blot analyses of the levels of proteins of iNOS and COX-2 were determined by electrophoresis on 4-12% SDS gradient polyacrylamide gels and then electrotransferred to Immobilon-P membranes. The membranes were probed with anti-iNOS and anti-COX-2, and then HRP-conjugated secondary antibody. (C) Gene expression of pro-inflammatory cytokines detected in RAW264.7 and LPS-induced RAW264.7 cells. The expression of GAPDH, the house keeping gene, was qualitatively detected by the presence of bands in all experiments. The results are representatives of at least three independent experiments.

For the upregulation of the level of specific mRNA transcripts for COX-2 and pro-inflammatory cytokines (IL-1β, IL-6 and TNF-α) in LPS-stimulated RAW264.7 cells, two critical transcription factors such as NF-kB and AP-1 should to be activated as downstream events of the TLR4-mediated inflammatory signaling pathway [10]. In addition, the activation of MAPKs (ERK, p38 MAPK, and JNK) is known to contribute to the TLR4-mediated inflammatory signaling pathway, leading to activation of NF-κB and AP-1 [3, 10, 23, 29]. To examine whether the MC fraction is able to inhibit LPS-induced activation of MAPKs, we further evaluated the effect of the MC fraction on the status of activating phosphorylation of MAPKs by Western blot analysis. As shown in Fig. 4, the activating phosphorylation of p38 MAPK, JNK, and ERK was significantly enhanced after stimulation of RAW264.7 cells with LPS. However, under these conditions, the presence of the MC extract appeared to reduce LPS-induced their activating phosphorylation in a dose-dependent manner.

Consequently, these results demonstrated that LPS-induced activation of MAPKs (p38 MAPK. JNK, and ERK) in RAW 264.7 cells were suppressed by the anti-inflammatory action of MC extract of *Sorghum bicolor* (L.) Moench var. Hwanggeumchal grains.

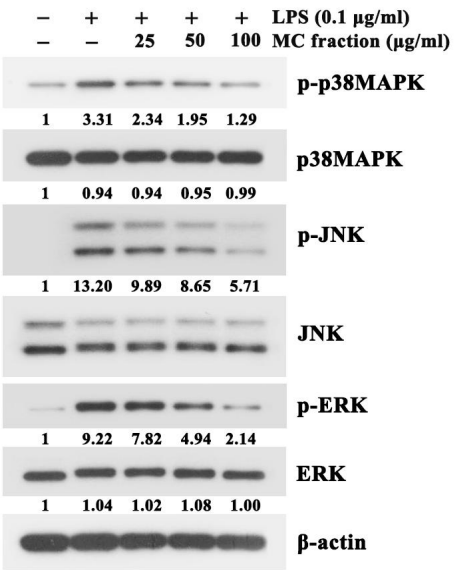

Fig. 4. Effect of the MC fraction on LPS-induced MAPK activation. RAW264.7 cells were pre-treated for 1 hr with indicated concentrations of the MC fraction and then treated with LPS (0.1 μg/ml) for 20 hr. And then the expression and activation of p38 MAPK, c-Jun, JNK, and ERK were assessed by Western blot analysis using antibodies specific for either total kinases or phosphorylated forms of kinases. The results are representatives of at least three independent experiments.

Identification of anti-inflammatory compounds in the MC fraction from Hwanggeumchal grains

In order to identify the components that predominantly exert the anti-inflammatory activity of the MC fraction, we analyzed the phenolic compounds within the MC fraction using HPLC. As a result, gentisic acid (8.73 μg/mg extract) and naringenin (4.50 μg/mg extract), among the phenolic compounds that have been previously reported to possess anti-inflammatory activity, appeared to account for 51.3% of the entire phenolic compounds in the MC fraction.

To elucidate whether gentisic acid and/or naringenin are responsible for the anti-inflammatory activity of the MC fraction, we intended to compare the 80% EtOH fraction (25, 50, and 100 μg/ml), gentisic acid (50, 100, and 200 μM), and naringenin (50, 100, and 200 μM) for their anti-NO production activities in LPS-stimulated RAW264.7 cells. As shown in Fig. 5, gentisic acid or naringenin at a concentration

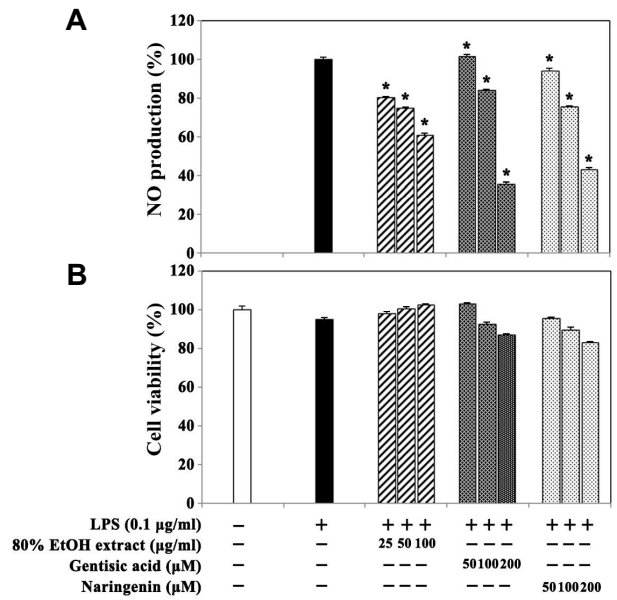

Fig. 5. Comparisons of anti-inflammatory activity of the MC fraction with gentisic acid and naringenin on LPS-induced RAW264.7cells. Cells were pre-incubated for 1 hr with the MC fraction (25, 50 and 100 μg/ml), gentisic acid or naringenin (50, 100 and 200 μM) in triplicate and then treated with LPS (0.1 μg/ml) for 20 hr. The culture supernatants were saved and used to determine NO production. MTS were employed to check the cell viability. Each value is expressed as mean ± SD (n=3 with six replicates per independent experiment). $p<0.05$ compared with control.

of 50 μM (correspond to 7.7 μg/ml for gentisic acid or 13.6 μg/ml for naringenin) was able to reduce the LPS-induced NO production to the level of 54.7% or 38.7%, respectively. These results demonstrated that the anti-inflammatory activity of the MC fraction might be mainly exerted by its phenolic components (gentisic acid and naringenin). In the literature, gentisic acid has several physiological activities including anti-oxidative and anti-tumor activities [31], anti-fibroblast growth factor [9], and LDL peroxidation suppression and anti-inflammatory effects [17]. Naringenin is known to be a kind of flavonoid possessing anti-inflammatory and anti-tumorigenic activity and is found in fruits and vegetables [13]. The anti-inflammatory activity of naringenin, which was evaluated in LPS-stimulated murine microglial BV-2 cell model, was mainly attributable to inhibition of NF-kB activation [28]. Besides these two phenolic compounds, hesperitin, kaempferol, salicylic acid, and quercetin, all of which were previously reported to have anti-inflammatory activity, were also detected as minor components in the MC fraction.

In conclusion, the grains of *Sorghum bicolor* (L.) Moench var. Hwanggeumchal are an excellent candidate for functional food source in prevention and treatment of inflammation-related diseases.

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The Conflict of Interest Statement

The authors declare that they have no conflicts of interest with the contents of this article.

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초록 : 지질다당류-자극된 마우스 대식세포주 RAW264.7에서 황금찰수수 종자의 항염증 활성

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황금찰수수 종자의 항염증 활성을 알아보기 위해, 국내에서 재배되고 있는 3가지 수수 품종(황금찰, 현찰, 찰)의 종자로부터 에탄올 추출물을 확보하여, 지질다당류(LPS)에 의해 자극된 마우스 대식세포주 RAW264.7를 이용하여 염증반응 관련 일산화질소(NO) 생성에 대한 억제 활성을 확인하였다. 세가지 품종 중에서 황금찰수수 종자의 억제활성이 가장 강하게 확인되었다. 황금찰수수 종자의 EtOH 추출물은 n-헥산, 메틸렌클로라이드(MC), 에틸아세테이트(EtOAc), 및 n-부탄올로 순차적으로 분획하였다. 이후 활성이 강하게 확인된 MC 분획을 이용하여, LPS-자극 RAW 264.7 세포에서 항염증 효능을 구체적으로 조사한 결과, LPS-자극에 의해 유도되는 염증반응관련 iNOS, COX-2 및 친염증성 사이토카인(IL-1β, IL-6, TNF-α)의 발현을 현저히 감소시켰다. 또한 MC 분획은 LPS-자극에 의해 염증관련 현상으로 유도되는 p38 mitogen-activated protein kinase (MPAK), c-Jun N-말단 키네이스(JNK) 및 세포외 신호조절 키네이스(ERK)의 활성화를 억제하는 것으로 나타났다. HPLC 분석으로 MC분획을 더 조사한 결과, 주요 구성 페놀화합물로서 gentisic acid와 naringenin을 확인하였다. Gentisic acid와 naringenin은 유사한 수준으로 LPS처리에 의해 RAW264.7 세포에서 유도되는 NO 생성에 대한 강력한 억제 활성을 나타내었다. 이상의 연구결과들은 황금찰수수 종자의 항염증 활성과 관련된 gentisic acid 및 naringenin의 작용을 잘 보여주며, 아울러 황금찰수수 종자가 곡물 기반 기능성 식품 개발에 적용될 수 있는 유익한 건강 효과를 가지고 있음을 시사한다.