ISSN (Print) 1225-9918
ISSN (Online) 2287-3406
Journal of Life Science **2020** Vol. 30. No. 5. 476~482 DOI : https://doi.org/10.5352/JLS.2020.30.5.476

- Note -

Eleocharis kuroguwai Ohwi Ameliorates LPS-mediated Inflammation by Suppressing MAPKs Signaling

Kang-Hoon Kim[1], Eun Young Bae[2], Hyun Goo Lim[3], Mi Heui Kim[4], Tae-Seok Oh[3]* and Seung Woong Lee[1]*

[1]*Immunoregulatory Material Research Center, Korea Research Institute of Bioscience and Biotechnology, 181 Ipsin-gil, Jeongeup-si, Jeonbuk 56212, Korea*
[2]*Elohim Co., Ltd, R&D Center, 199 Techno 2-ro (Yongsan-dong, Migun Technoworld 1-cha), Yuseong-gu, Daejeon 34025, Korea*
[3]*Department of Plant Resources, College of Industrial Science, Kongju National University, Yesan 32439, Korea*
[4]*Department of Agricultural Environment Rural Environment and Resources Division, National Institute of Agricultural Sciences, 166 Nongsaengmyeong-ro, lseo-myeon, Wanju-gun 55365, Korea*

Received March 21, 2020 /Revised May 6, 2020 /Accepted May 7, 2020

In rice agriculture, *Eleocharis kuroguwai Ohwi* (olbanggae) is a target for herbicidal intervention as a problem weed although it has also long been used clinically as a traditional medicine for jaundice, fever, and blood flow. *E. kuroguwai* has been evaluated in many clinical trials, but its molecular biological advantages are still unknown. Here, we investigate the anti-inflammatory effects of *E. kuroguwai* 80% ethanol extracts by screening NO production in LPS-induced macrophage activation. To find the most effective fractions, we partitioned five sub-fractions using HP20 column chromatography, namely 20%, 40%, 60%, 80%, and 100%. Of these, the 60% and 80% sub-fractions were found to significantly inhibit NO production; there were no toxicological effects at any concentration. In addition, the 80% sub-fraction inhibited significantly the iNOS and the mRNA of the pro-inflammatory mediators IL-6, TNF-α, and IL-1β by inhibiting the phosphorylation of JNK and ERK pathways associated with MAPKs signaling. Our results suggest that the 80% *E. kuroguwai* sub-fraction has the most significant anti-inflammatory effects of inhibiting iNOS and pro-inflammatory mediators and suppressing the phosphorylation of JNK and ERK. Therefore, the 80% sub-fraction of *E. kuroguwai* extract may be a therapeutic candidate for inflammatory diseases associated with the overexpression of MAPKs.

Key words : Anti-inflammation, *Eleocharis kuroguwai Ohwi,* iNOS, MAPK

Introduction

Inflammation is a biological response of the immune cells that can be triggered by a variety of factors, including pathogens, damaged cell and toxins, and be act by removing injurious stimuli and initiating the healing process [2]. Nitric oxide (NO) is involved in a novel signaling molecule in the inflammatory pathogenesis. NO production requires by three nitric oxide synthase (NOS), which catalyze the oxidation of L-arginine to L-citrulline; endothelial NOS, neuronal NOS and inducible NOS (iNOS) [18]. Though NO leads an anti-inflammatory effect under normal physiological conditions, NO is considered as pro-inflammatory mediator that causes inflammation owing to over production in abnormal conditions [6]. In inflammatory action, activated macrophages express transcriptionally and translationally iNOS and results from profound NO production [20].

Pro-inflammatory mediators such as interleukin-6 (IL-6), interleukin-1beta (IL-1beta), and tumor necrosis factor alpha (TNF-alpha) lead to inflammatory signaling associated with important intracellular signaling pathways [1]. Among several pathways, mitogen-activated protein kinases (MAPKs), which are family of serine/threonine protein kinase, regulate cell proliferation, differentiation, survival and apoptosis [7]. The mammalian MAPKs include extracellular-signal-regulated kinase (ERK) 1/2, p38 MAP Kinase, and c-Jun N-terminal kinases (JNK) [8]. Activation of the MAPKs, including Erk1/2, JNK, leads to phosphorylation and activation of p38 transcription factors present in the cytoplasm or nucleus, which initiates the inflammatory response [16]. If

***Corresponding authors**
Tel : +82-41-330-1200, Fax : +82-41-330-1209
E-mail : ots1022@kongju.ac.kr (Tae-Seok Oh)
Tel : +82-63-570-5264, Fax : +82-63-570-5239
E-mail : lswdoc@kribb.re.kr (Seung Woong Lee)

inhibitory agents of phosphorylation of MAP kinases among natural substituents were developed, safe treatment will be possible for inflammatory diseases.

Eleocharis kuroguwai Ohwi (*E. kuroguwai*) is a perennial herb in the sedge family, Cyperaceae, and lives in pond, wet land, and rice paddy [15]. Especially, *E. kuroguwai* proliferates rapidly to cover rice fields and then, compete with rice growth [5]. Thus, *E. kuroguwai* became a dominant problem weed because of wide use of herbicides in rice cultivation [10]. To effectively eliminate *E. kuroguwai,* researches, related to physio-ecological and tuberization characteristics has been studied [9]. In Korean traditional medicine, however, *E. kuroguwai* has consumed for chaste and sweet flavor grocery as an emergency food, and used for jaundice, fever clearance, and hemagogue [4]. Despite medicinal effects of *E. kuroguwai,* molecular science-based biological effects were still unknown in *E. kuroguwai* extract. In our study, we first tested inhibitory effects of NO production under LPS-mediated inflammatory conditions in RAW 264.7 using *E. kuroguwai* extracts (total EtOH extract and sub-fractions). In addition, we further evaluated pro-inflammatory cytokines; iNOS, IL-6, TNF-α, and IL-1β and MAPKs pathway; phosphorylation of JNK, ERK, and p-38. Our results suggested that 80% sub-fraction of *E. kuroguwai* extract of would be safe therapeutic agent for inflammatory diseases.

Materials and Methods

Plant material and Extraction

Eleocharis kuroguwai Ohwi were collected from Yesangun, Chungnam, Korea in July 2019. The plant was identified by Professor Chang Ho Kim (Kongju National University), and a voucher specimen (no. 20190123) was deposited at the Kongju National University. Aerial parts of *Eleocharis kuroguwai* Ohwi were air-dried, pulverized to smaller than 1 cm size. The dried plant material (193 g) were extracted with 80% EtOH (300 l) at 70℃ for 3 hr, and the above process was repeated twice. After filtering (No. 10, 185 mm, Hyundai micro Co., Seoul, Korea) the extract *in vacuo*, the filtrates were concentrated by evaporation to obtain 49 g of extract. The extract were suspended in distilled water (2 l), and it was subjected to HP20 gel column chromatography and eluted with a step-wise gradient of increasing MeOH in H_2O (0:100, 20:80, 40:60, 60:40, 80:20, and 100:0, *v*/*v*) to obtain 6 sub-fractions. The extract and these fractions were measured for anti-inflammatory activity.

Cell cultures

RAW264.7 (ATCC TIB-71) cells was cultured in Dulbecco's modified Eagle medium (DMEM) and RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 100 U/ml penicillin, and 100 mg/ml streptomycin sulfate. Cell were maintained at 37℃ in humidified air with 5% CO_2 [12].

Measurement of NO contents and cell cytotoxicity

RAW264.7 (ATCC TIB-71) cells was cultured in Dulbecco's modified Eagle medium (DMEM) and NO assay was carried out for measurements of NO release using previously reported method [12]. Briefly, RAW264.7 cells were plated at 1×10^5 cell density in 96-well microplate, and cultured for 24 hr. *E. kuroguwai* extracts were pretreated with increasing dose concentrations (10, 30, 60, or 90 μg/ml), and than stimulated with LPS (1 μg/ml, Sigma–Aldrich, St. Louis, MO, USA) for 18 hr. The mixture of cell supernatant (100 μl) and Griess reagent [(1% sulfanilamide + 0.1% N-(1-naphthyl)ethylenediamine (Sigma–Aldrich, St. Louis, MO, USA)] in 5% phosphoric acid was recorded at 550 nm using a microplate reader (Varioskan LUX, Thermo Fisher Scientific Inc., Waltham, MA, USA). RAW264.7 cell cytotoxicity was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [13].

Real-time PCR using TaqMan Probe

Total RNA was extracted from RAW 264.7 cells using the TaKaRa MiniBEST Universal RNA Extraction Kit following the manufacturer's instructions (TaKaRa Bio Inc., Shiga, Japan). The complementary DNA (cDNA) was synthesized from 1 μg of the total RNA using a PrimeScript 1st strand cDNA synthesis kit (Takara Bio Inc. Japan). Quantitative real-time PCR (qPCR) of Il1bβ (Mm00434228_m1), Il6 (Mm 00446190_m1), and Tnf (Mm00443258_m1) was performed with a TaqMan Gene Expression Assay Kit (Thermo Fisher Scientific, San Jose, CA, USA). To normalize the gene expression, an 18S rRNA endogenous control (Applied Biosystems, Foster City, CA, USA) was used. The qPCR was employed to verify the mRNA expression using a Step-One Plus Real-Time PCR system. To quantify mRNA expression, TaqMan mRNA assay was performed according to the manufacturer's protocol (Applied Biosystems) [3]. PCR amplification was analyzed using the comparative △△CT method.

Immunoblots analysis

The whole cell lysate was extracted using Cell Lysis Buffer (Cell Signalling Technology, Beverly, MA, USA). Immunoblots analysis was performed as previously described method [11]. After transfer to nitrocellulose (NC) membrane, the blocking membrane with 5% skimmed milk powder was incubated overnight at 4℃ with primary antibody, including anti-iNOS (1:1,000), anti-phospho-JNK (1:1,000), anti-phospho-p38 (1:1,000), anti-phospho-ERK (1:1,000), and anti-β-actin antibodies (Cell Signalling, Beverly, MA, USA). The membranes were then incubated with a horseradish peroxide-conjugated anti-rabbit secondary antibody (1:5,000) at room temperature. The band densities were calculated with Quantity One software. (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

GraphPad Prism 5 software (GraphPad software, San Diego, CA, USA) was used for the statistical analysis of the experimental results. Each experiments, including NO assay, MTT assay, Immunoblots and real-time PCR, were performed independently three times, and these data represent the mean ± SEM. The statistical significance of each value was measured by the unpaired Student's t test. *p<0.05, **p<0.01, and ***p<0.001 were considered significant.

Results and Discussion

Inhibitory effects of nitric oxide production from the *E. kuroguwai* extract and sub-fraction

NO is a major pro-inflammatory mediator, involved in the pathogenesis of inflammation [17]. A previous study reported that *E. kuroguwai* extract has used for jaundice and antifebrile in traditional Korean medicine. However, the efficacy of *E. kuroguwai* extract-based on molecular science was still uncovered. To investigate inhibitory action of NO associated inflammatory effects, we evaluated the NO production in LPS-induced RAW 264.7 cells, a mouse macrophage cell line, upon treatment with the *E. kuroguwai* 80% EtOH extract (Fig. 1A). On 30 and 60 μg/ml of *E. kuroguwai* 80% EtOH extract, NO production was significantly inhibited (Fig. 1A). To evaluate most effective fractions of an-

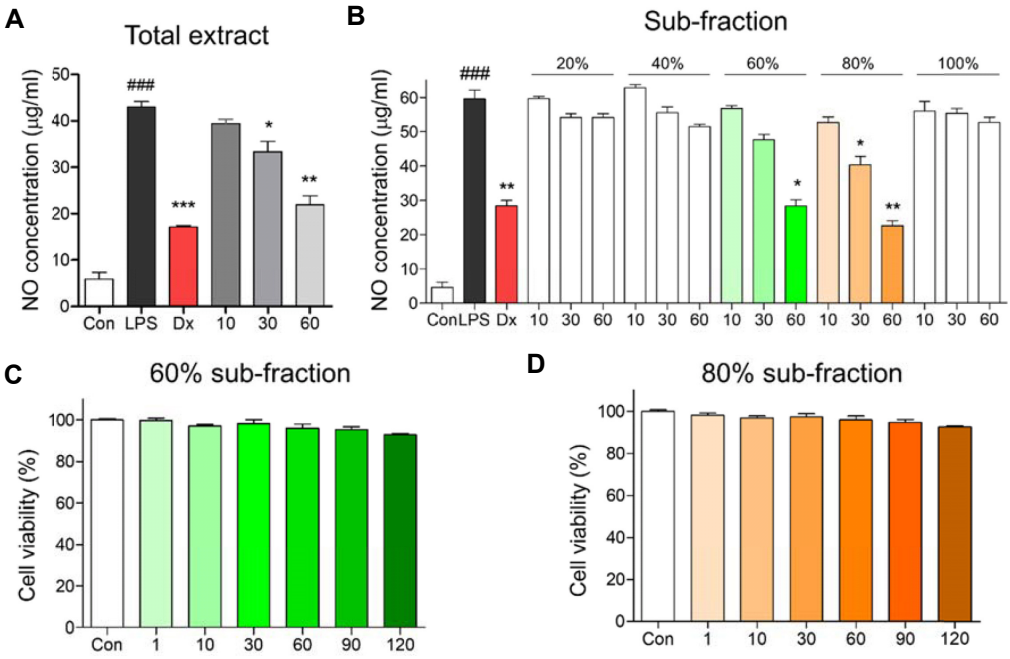

Fig. 1. *E. kuroguwai* extract and sub-fractions inhibited nitric oxide production in LPS-induced RAW 264.7 cell line. (A) NO concentration evaluated in *E. kuroguwai* total extract. NO evaluation performed triplicate test, and results described as Mean ± SEM. An unpaired Student's *t* test was used for statistical analysis. ###p<0.001 versus Con. *p<0.05, **p<0.01 versus LPS. Con; control, LPS; lipopolysaccharide, Dx; dexamethasone. (B) NO concentration evaluated in the 20% to 100% sub-fractions of *E. kuroguwai* extract. NO evaluation performed triplicate test, and results described as Mean ± SEM. An unpaired Student's *t* test was used for statistical analysis. ###p<0.001 versus Con. *p<0.05, **p<0.01 versus LPS. Con; control, LPS; lipopolysaccharide, Dx; dexamethasone. (C) Cell viability was evaluated in 1 to 120 mg/ml in the 60% sub-fraction. (D) Cell viability was evaluated in 1 to 120 mg/ml in the 80% sub-fraction.

ti-inflammation, furthermore, 80% EtOH sub-fraction of *E. kuroguwai* extract were subjects to HP20 gel column chromatography eluted with 0% to 100% ethanol and each sub-fractions tested in LPS-induced RAW 264.7 cells (Fig. 1B). The 60% (60 μg/ml) and 80% sub-fractions (30 and 60 μg/ml) significantly inhibited NO production in LPS-induced RAW 264.7 cells. Additionally, to investigate cellular toxicology after treatment of the 60% and 80% sub-fractions, we tested cell viability from 10 to 120 μg/ml using MTT assay (Fig. 1C). In the 60% and 80% sub-fractions, there were no toxicological effects without below 120 μg/ml. These results suggested that *E. kuroguwai* extracts had anti-inflammatory effects and that the major bioactivity of inhibitory NO production in each fractions is the 80% sub-fraction.

The 80% sub-fraction inhibits iNOS, and pro-inflammatory cytokines

To evaluate biological molecular evidences of effectively reduced NO production treated with the 80% sub-fraction, we evaluated pro-inflammatory mediators such as iNOS, TNF-α, IL-6, and IL-1β under LPS-induced macrophage, RAW 264.7 cell line (Fig. 2). Because iNOS, which is involved in production of NO, induction is novel signalling molecule associated with MAPKs pathway [19]. Subsequently, pro-inflammatory cytokines such as, IL-6, TNF-α, and IL-1β are induced by MAPK signalling pathway [14]. Under non-toxic doses of cellular viability, we observed immunoblot of iNOS expression after treatment with the 80% sub-fraction of 10, 30, 60, and 90 μg/ml (Fig. 2A). In our results, the 80% sub-fraction (90 μg/ml) showed significant inhibition of iNOS under LPS-induced macrophage on Western blot and its graph (Fig. 2B). Moreover, pro-inflammatory cytokines; IL-6, TNF-α, and IL-1β, were significantly disturbed after treatment with 80% sub-fraction of 90 μg/ml (Fig. 2C, Fig. 2D, Fig. 2E). These results suggested that the 80% sub-fraction (90 μg/ml) had anti-inflammatory effects by inhibition of iNOS, IL-6, TNF-α, and IL-1β.

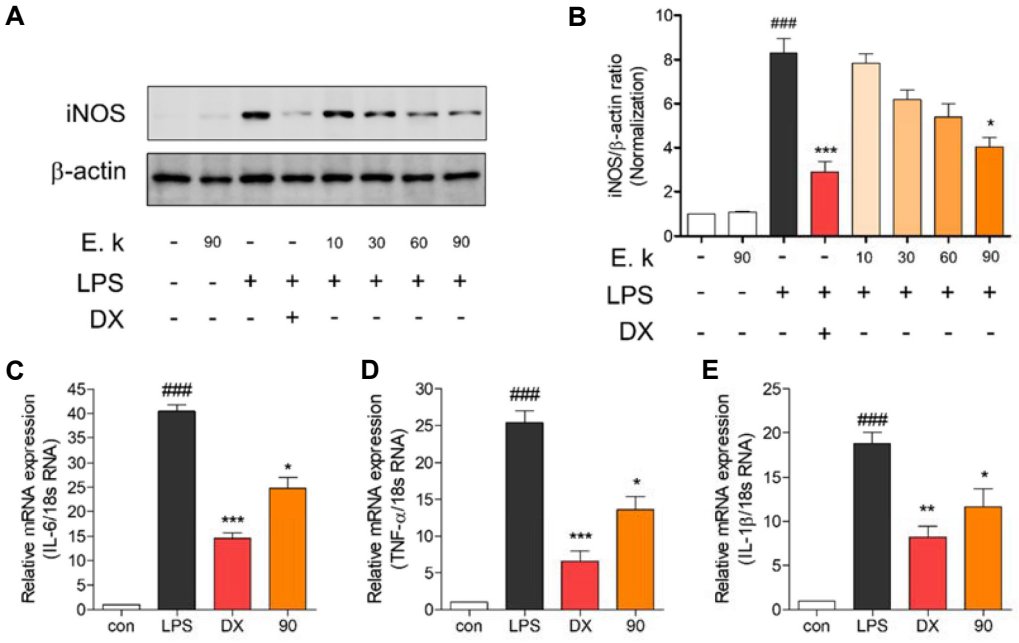

Fig. 2. The 80% sub-fraction showed anti-inflammatory effects by inhibiting pro-inflammatory mediators. (A) The 80% sub-fraction decreased iNOS expression levels in LPS-induced RAW 264.7 cells. (B) Relative ratio of iNOS versus β-actin was measured using densitometry, and dexamethasone was used as positive control. These graphs represented that the 80% sub-fraction dose-dependently inhibited iNOS using immunoblot analysis. Cells were pretreated with each compound for 2 hr and stimulated with LPS (1 μg/ml) for 16 hr. Immunoblot analysis was performed in triplicate tests, and results are expressed as means ± SEM. An unpaired Student's t-test was used for statistical analysis. ###p<0.001 versus Con, *p<0.05, **p<0.01, and ***p<0.001 versus LPS. (C – E) The mRNA expression levels of IL-6, TNF-α, and IL-1β were measured using quantitative real-time PCR experiment, and these proinflammatory cytokines were significantly diminished by the 80% sub-fraction. Cells were preincubated for 2 hr with the 80% sub-fraction (90 μg/ml), and activated by LPS (1 μg/ml) for 2 hr. Results represented as mean ± SEM, and dexamethasone was used as a positive control. ###p<0.001 versus Con, *p<0.05, **p<0.01, and ***p<0.001 versus LPS. Con: control, LPS: lipopolysaccharide, Dx: dexamethasone.

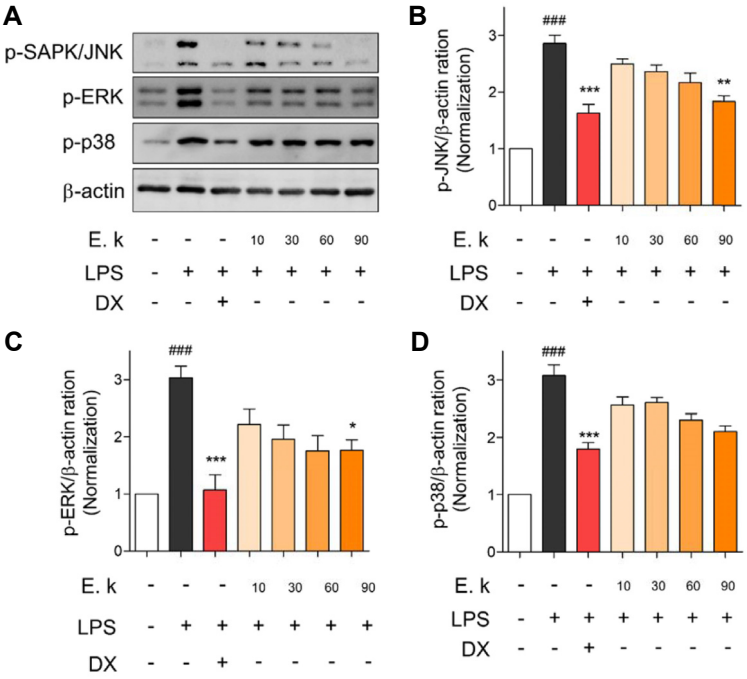

Fig. 3. The 80% sub-fraction suppressed MAPK signalling pathway. (A) Immunoblot analysis showed that phosphorylated JNK, ERK, and p38 of MAPKs pathway were observed after treatment with the 80% sub-fraction in RAW 264.7 macrophages. (B – D) The graphs represent ratio of protein level of JNK (B), ERK (C), and p38 (D). Cells were pre-incubated for 2 hr with the 80% sub-fraction (10, 30, 60, and 90 μg/ml), and stimulated with LPS (1 μg/ml) for 1 hr. Dexamethasone served as positive control. Immunoblot analysis performed as triplicate experiments, and data represented as means ± SEM. Significant difference was considered at the levels of ###p<0.001 versus Con, *p<0.05, **p<0.01, and ***p<0.001 versus LPS. Con: control, LPS: lipopolysaccharide, Dx: dexamethasone.

The 80% sub-fraction disrupted JNK and ERK signalling under LPS-induced inflammation

To investigate inhibition of pro-inflammatory mediators under LPS-induced inflammation, we evaluate major inflammatory signalling associated with JNK, ERK, and p38 using Western blotting (Fig. 3). Under non-toxic doses of cellular viability, we observed phosphorylation of JNK, ERK, and p38 expression after treatment with the 80% sub-fraction of 10, 30, 60, and 90 μg/ml. In results of Western blotting, the 80% sub-fraction (90 μg/ml) significantly inhibited phosphorylation of JNK and ERK (Fig. 3A). The protein expression ratios of phosphorylation of JNK, ERK, and p38 were shown in Fig. 3B, Fig. 3C, Fig. 3D. These results suggested that the 80% sub-fraction (90 μg/ml) had anti-inflammatory effects by disruption of phosphorylation of JNK and ERK under LPS-induced inflammation. Through these results, *E. kuroguwai* extract partly coved an anti-inflammatory effects by inhibiting MAPK pathways. These results indicate that *E. kuroguwai* extract and its 80% sub-fraction, may be useful and safe therapeutic agents for treatment with inflammatory diseases with minimal side effects. Further studies are necessary to improve isolation of natural compounds among *E. kuroguwai* 80% sub-fraction extract using separation analysis and determine bioactivities of its substituents under inflammatory model.

E. kuroguwai extract, which has been treated for jaundice, fever clearance, and hemagogue in traditional Korean medicine. In these results, we found the most effective inhibition of fraction concentration, the 80% sub-fraction among 0% to 100% sub-fractions. In addition, we determined that the 80% sub-fraction had anti-inflammatory effects by inhibition of the major intracellular inflammatory signaling pathways associated with MAPKs and amelioration of pro-inflammatory mediators such as iNOS, IL-6, TNF-α, and IL-1β. These results indicate that the 80% sub-fraction may be useful and safe therapeutic agents for inflammatory action such as rheumatoid arthritis, allergic asthma, and atopic dermatitis. Through anti-inflammatory effect of *E. kuroguwai* extract and the 80% sub-fraction, further researches should be proved under safe and therapeutic evaluation of pre-clinical and clinical studies.

Acknowledgements

This work was supported by a grant from the KRIBB Research Initiative Program (KGS1002012) and the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (2017-0880-03).

The Conflict of Interest Statement

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

References

1. Abdulkhaleq, L. A., Assi, M. A., Abdullah, R., Zamri-Saad, M., Taufiq-Yap, Y. H. and Hezmee, M. N. M. 2018. The crucial roles of inflammatory mediators in inflammation: A review. *Vet. World* **11**, 627-635.
2. Ashley, N. T., Weil, Z. M. and Nelson, R. J. 2012. Inflammation: Mechanisms, costs, and natural variation. *Annu. Rev. Ecol. Evol. S.* **43**, 385-406.
3. Bertucci, A., Kim, K. H., Kang, J., Zuidema, J. M., Lee, S. H., Kwon, E. J., Kim, D., Howell, S. B. and Ricci, F. 2019. Tumor-targeting, microrna-silencing porous silicon nanoparticles for ovarian cancer therapy. *ACS. Appl. Mater. Inter.* **11**, 23926-23937.
4. Chul, C., Rhee, I. S., Lee, S. K. and Kang, S. A. 2008. A study on the qualitative properties of traditional sake using allbanggae. *J. Kor. Soc. Food. Sci. Nutr.* **37**, 784-791.
5. Chung, I. M., Kim, J. T. and Kim, S. H. 2006. Evaluation of allelopathic potential and quantification of momilactone a,b from rice hull extracts and assessment of inhibitory bioactivity on paddy field weeds. *J. Agric. Food. Chem.* **54**, 2527-2536.
6. Coleman, J. W. 2001. Nitric oxide in immunity and inflammation. *Int. Immunopharmacol.* **1**, 1397-1406.
7. Kaminska, B. 2005. Mapk signalling pathways as molecular targets for anti-inflammatory therapy--from molecular mechanisms to therapeutic benefits. *Biochim. Biophys. Acta.* **1754**, 253-262.
8. Kim, E. K. and Choi, E. J. 2010. Pathological roles of mapk signaling pathways in human diseases. *Biochim. Biophys. Acta.* **1802**, 396-405.
9. Kim, H. D., Park, J. S., Park, K. S., Choi, Y. J., Yu, C. J., Shim, S. W., Rho, Y. D. and Kwon, Y. W. 1997. Studies on tuberization characteristics of water chestnut (*Eleocharis kuroguwai ohwi*). *Kor. J. Weed. Sci.* **17**, 10-23.
10. Kim, H. D., Park, J. S., Park, K. Y., Choi, Y. J., Yu, C. J., Rho, Y. D. and Kwon, Y. W. 1996. Studies on characteristics of sprouting and occurrence on paddy field of water chestnut (*Eleocharis kuroguwai ohwi*). *Kor. J. Weed. Sci.* **16**, 264-281.
11. Kim, K. H., Lee, I. S., Park, J. Y., Kim, Y., An, E. J. and Jang, H. J. 2018. Cucurbitacin b induces hypoglycemic effect in diabetic mice by regulation of amp-activated protein kinase alpha and glucagon-like peptide-1 via bitter taste receptor signaling. *Front. Pharmacol.* **9**, 1071.
12. Kim, K. H., Park, E. J., Jang, H. J., Lee, S. J., Park, C. S., Yun, B. S., Lee, S. W. and Rho, M. C. 2019. 1-Carbomethoxy-β-carboline, derived from *Portulaca oleracea* L., ameliorates lps-mediated inflammatory response associated with mapk signaling and nuclear translocation of NF-κb. *Molecules* **24**, 4042.
13. Lim, H. J., Jang, H. J., Bak, S. G., Lee, S., Lee, S. W., Lee, K. M., Lee, S. J. and Rho, M. C. 2019. In vitro inhibitory effects of cirsiliol on IL-6-induced STAT3 activation through anti-inflammatory activity. *Bioorg. Med. Chem. Lett.* **29**, 1586-1592.
14. Liu, T., Zhang, L., Joo, D. and Sun, S. C. 2017. NF-κb signaling in inflammation. *Signal. Transduct. Target. Ther.* **2**, e17023.
15. Moon, B. C., Cho, S. H., Kwon, O. D., Lee, S. G., Lee, B. W. and Kim, D. S. 2010. Modelling rice competition with *Echinochloa crus-galli* and *Eleocharis kuroguwai* in transplanted rice cultivation. *J. Crop. Sci. Biotechnol.* **13**, 121-126.
16. Pearson, G., Robinson, F., Beers Gibson, T., Xu, B. E., Karandikar, M., Berman, K. and Cobb, M. H. 2001. Mitogen-activated protein (map) kinase pathways: Regulation and physiological functions. *Endocr. Rev.* **22**, 153-183.
17. Sharma, J. N., Al-Omran, A. and Parvathy, S. S. 2007. Role of nitric oxide in inflammatory diseases. *Inflammopharmacology* **15,** 252-259.
18. Sonar, S. A. and Lal, G. 2019. The iNOS activity during an immune response controls the cns pathology in experimental autoimmune encephalomyelitis. *Front. Immunol.* **10**, 710.
19. Tripathi, P., Tripathi, P., Kashyap, L. and Singh, V. 2007. The role of nitric oxide in inflammatory reactions. *FEMS. Immunol. Med. Mic.* **51**, 443-452.
20. Zhou, H. Y., Shin, E. M., Guo, L. Y., Youn, U. J., Bae, K., Kang, S. S., Zou, L. B. and Kim, Y. S. 2008. Anti-inflammatory activity of 4-methoxyhonokiol is a function of the inhibition of iNOS and COX-2 expression in RAW 264.7 macrophages via NF-κb, JNK and p38 mapk inactivation. *Eur. J. Pharmacol.* **586**, 340-349.

초록 : LPS로 유도된 염증 상태 내에서 MAPKs 세포신호 전달체계를 저해하는 올방개의 항염증 효과

김강훈[1] · 배은영[2] · 임현구[3] · 김미희[4] · 오태석[3]* · 이승웅[1]*
([1]한국생명공학연구원 면역조절소재연구센터, [2]엘로힘 R&D 센터, [3]공주대학교 식물자원학과, [4]국립농업과학원 농림환경자원부)

벼 농사에서 마름으로 불리는 올방개는 문제의 잡초 종류 중 하나로서 제초 작용의 대상이다. 하지만, 올방개는 임상적으로 황달, 해열 및 통경을 위한 전통 의학에 쓰여왔다. 올방개는 오랫동안 임상적으로 사용되어왔음에도 불구하고, 분자생물학 기반의 생물학적 효과는 아직 밝혀지지 않았다. 때문에, 본 연구진은 LPS로 유도된 대식세포 내에서 올방개 추출물 처리에 의한 NO 생산량을 스크리닝 함으로써 항염증 효과를 조사했다. 가장 효과적인 항염증 fraction을 찾기 위해, 본 연구진은 HP20 column chromatography를 이용하여 5 종류의 EtOH sub-fraction을 동정하였다; 20%, 40%, 60%, 80%, 및 100% EtOH. 동정한 sub-fraction 중에서, 60%와 80% 성분에서 가장 유의성 있는 NO 생산의 저해를 나타냈으며, 세포독성 효과도 나타내지 않는 결과를 나타냈다. 추가적으로 80% sub-fraction은 유의성 있는 iNOS 저해와 전염증성 매개체의 mRNA; IL-6, TNF-α, 그리고 IL-1β를 저해하였으며, 이 결과는 MAPKs 세포신호전달과 관련 있는 JNK 및 ERK 단백질의 인산화 저해가 원인임을 밝혔다. 본 연구진의 결과는 올방개 추출물 80% sub-fraction EtOH가 항염증 활성을 가장 효과적으로 저해하는 성분 임을 밝혔으며, 이는 MAPKs 세포신호전달의 과 발현에 의한 염증성 질병의 치료제 후보 성분 일 수 있음을 시사한다.