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Anti-inflammatory activities of polyphenols from *Oryza sativa* L.

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ABSTRACT

Leaves and stems of *Oryza sativa* L. were extracted and fractionated to get polyphenols.

The polyphenol fraction inhibited the induction of NO by inhibition of the expression of mRNA of iNOS and protein induced by LPS. With ELISA analysis, the polyphenol fraction was found to inhibit the expression extra-cellular inflammatory cytokines, IL-1β, IL-6, TNF-α as well as their mRNAs. This anti-inflammatory activity of polyphenols was proved by blocking the MAPK pathway by inhibition of phosphorylation of ERK1/2, JNK, p38.

KEYWORDS

Oryza sativa L.;
Polyphenols;
Anti-inflammation;
MAPK pathway;
Cytokine.

INTRODUCTION

Oryza sativa L. has been used as the major food resource in Asia. Only a small part of *Oryza sativa* L. has been used as rice, the seed or seed core. Other parts, however, have been wasted as fertilizer or used for traditional purposes. Scientific researches for other parts of rice, leaf, stem and root of *Oryza sativa* L. have been required to develop the possible use of huge natural resources wasted.

Recently *Oryza sativa* L. has been reported it has various bioactive compounds. It has antioxidant activity[1,2], antitumor activity[3], antimicrobial activity[4], tyrosinase inhibition activuity for skin whitening[5,6,7]. Some bioactive compounds from the rice were reported as steroids[8], terpenoids, and alkaloids. Some of compounds were extracted from the seed coat. 1-tetratriacontanol, β-sitosterol, momilactone A, momilactone B, tricin were separated. Among them, momilactone B was reported its anticancer activities[9,10,11].

Polyphenols have been known as strong antioxidants[2]. Antioxidants containing polyphenolic substructure are known more than 4,000 distinct species. They have strong antioxidant activity in vitro as well as in vivo. They may also affect cell-to-cell signaling, receptor sensitivity, inflammatory enzyme activity.

This study was carried out to evaluate the anti-inflammatory efficacy of *Oryza savita* L. Aerial parts of *Oryza savita* L. was extracted and fractionated to get the polyphenol fraction. MTT assay was conducted to identify the toxicity of the fraction. After that, nitrate deducing activity was analyzed with mouse macrophage cell (Raw 264.7). Expressions of iNOS, COX-2 proteins were finally analyzed to evaluate the anti-inflammatory activity of polyphenol fraction of *Oryza savita* L.

EXPERIMENTAL

Materials

Oryza sativa L. used in this research was Korean ChuJung rice. It was collected in August 2012 from Ichon-city Korea. Silica gel for column chromatography was Kiesel gel 60(Merck, Darmstadt, Germany). Octadecyl silica gel(ODS) was Lichroprep RP-18(Merck). Thin layer chromatographywas done with TLC silica gel 60 F254(Merck) and TLC silica gel 60 RP-18 F254S(Merck). Infrared(IR) spectrum was made with Perkin model 599B (Buckinghamshire, England). Fast atom bombardment (FAB) mass spectrumwas made with JMSAX 700(JEOL).

Extraction and Fractionation

8.35 kg of leaves and stem of Oryza sativa L. was extracted with 80% MeOH for 24 hours at room temperature. Extract was filtered and vacuum evaporated to get the concentrate. The concentrate was fractionated with n-hexane (3 L×3) and H_2O(3 L). The H2O fraction was partitioned with ethyl acetate (EtOAc, 3 L×3). Again the H2O fraction was fractionated with n-butanol (n-BuOH, 3 L×3) and vacuum evaporated to concentrate. Again the concentrate portioned into 3 fractions, n-hexane(135 g, OSH), EtOAc (38 g, OSE), n-BuOH (26 g, OSB) and H_2O fraction.

MTT assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is used to determine cytotoxicity of polyphenol fraction(ethyl acetated fraction) for the viability and growth of with HaCat (ACTT, CLS 300493, USA). 100 µl of experimental cell solution was plated into each well of 96-well culture plate and incubated for 24 h in 5% CO2 incubator. After treatment of cells for 48 h by coffee extracts at 1; 5; 10; 50 and 100 mg/L in DMEM containing 1% AA, experimental media are removed and the cells are incubated with 50 µl basal medium containing 2.0 mg/ml MTT in CO2 incubator at 37 °C for 3 h. The medium is aspirated, and the formazan product is solubilized with 200 ul dimethyl sulfoxide (DMSO) every well. Absorbance at 595 nm was measured for each well using microplate absorbance reader.

Anti-inflammatory assay

The inhibitory activity of NO generation and cell toxicity were measured. The nitrate deduction activity was analyzed. MTT assay was made with mouse macrophage cell (Raw 264.7). Expressions of inflammatory proteins such as iNOS, COX-2 were measured with Western blot.

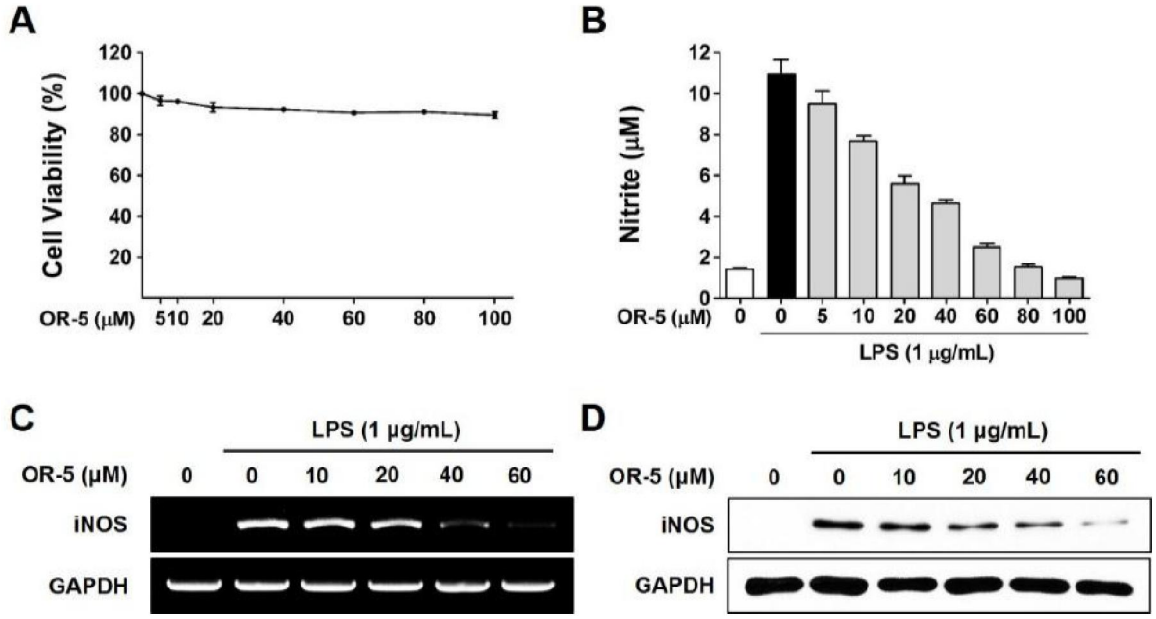

Figure 1 : Effects of polyphenol fraction(OR-5) on (A) cytotoxicity, (B) inhibitory NO induction, (C) iNOS mRNA, (D) inhibitory protein expression

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RESULTS AND DISCUSSION

Leaves and stems of *Oryza sativa* L. were extracted with 80% MeOH. The extract concentrated was fractionated by the sequential partitioning with EtOAc, n-BuOH, and H_2O. The EtOAc fraction was partly purified with silica gel and ODS column chromatography to get the polyphenol enriched fraction.

The inhibitory activity of polyphenol against NO induced by lipopolysaccharide (LPS) was measured. According to the MTT test, the polyphenol fraction didn't show any cytotoxicity up to 100 μM(Figure 1A). The polyphenol fraction inhibited the induction of NO by LPS. The inhibitory activity was dependent on concentration(Figure 1B). From results shown in Figure 1, it was proved that the polyphenol fraction inhibited the expression of mRNA of iNOS and protein induced by LPS.

We also found that the polyphenol fraction inhibited the expressions mRNAs for inflammatory cytokines, IL-1β, IL-6, TNF-α(Figure 2A). With ELISA analysis, the polyphenol fraction was found to inhibit the expression extra-cellular inflammatory cytokines as well as their mRNAs as shown in Figure 2B.

To find out the inflammatory mechanism of the polyphenol fraction, MAPK signal transfer pathway was monitored with western blot. MAPK pathway has been known as a major inflammatory signal transfer mechanism[12,13]. As shown in Figure 3, the polyphenol block the pathway by inhibition of phosphorylation of ERK1/2, JNK, p38. It was true because the inhibition was concentration dependent.

From this study, we found the polyphenol frac-

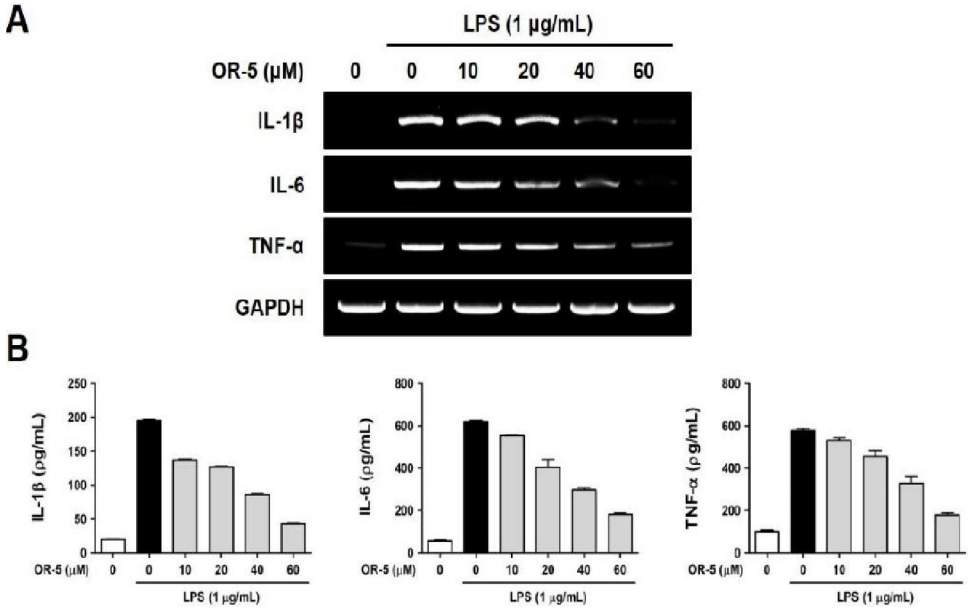

Figure 2 : Effects of polyphenol fraction(OR-5) on (A) inflammatory cytokine mRNAs (B) expression of proteins, IL-1β, IL-6, TNF-α

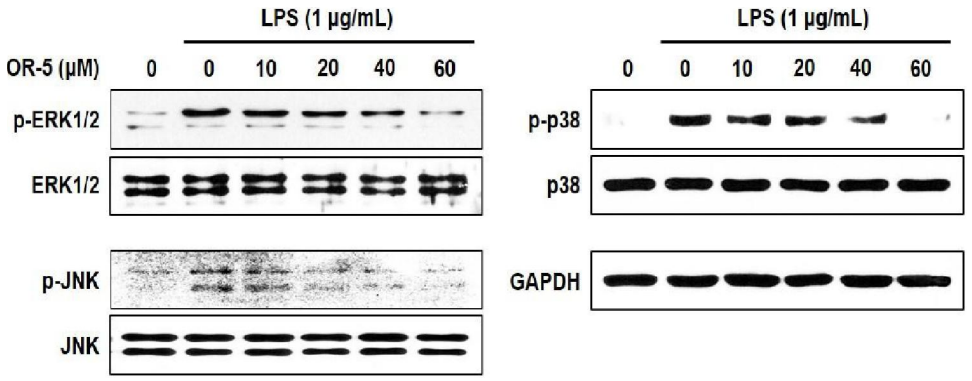

Figure 3 : Effects of polyphenol fraction(OR-5) on cellular MAPK signal transfer pathway

tion from *Oryza sativa* L. has anti-inflammatory activity. This was clear because polyphenols were proved to block the block the MAPK pathway by inhibition of phosphorylation of ERK1/2, JNK, p38. This study is meaningful because it suggests the possible use if bioactive compounds from rice. Rice has been used as one of the most important crops for several hundred years. Nowadays, however, the consumption of rice has been decreased continuously by changes of food consumption patterns. This change caused serious over production with problems in rural economy. It has been required to increase the value of rice in many ways. This study shows that the polyphenols in rice, *Oryza sativa* L., are bioactive compounds. Anti-inflammatory activity of polyphenols from *Oryza sativa* L. is the evidence of the possibility for further use of rice.

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