Appl Biol Chem (2016) 59(5):747–754
DOI 10.1007/s13765-016-0220-5

Online ISSN 2468-0842
Print ISSN 2468-0834

ARTICLE

Anti-inflammatory effects of luteolin and luteoloside from *Taraxacum coreanum* in RAW264.7 macrophage cells

Ah Young Lee[1] · Sullim Lee[2] · Hyun Young Kim[3] · Sanghyun Lee[4] · Eun Ju Cho[1]

Received: 4 July 2016 / Accepted: 10 August 2016 / Published online: 19 August 2016

Abstract The effects of luteolin (LT) and luteoloside (LS) from *Taraxacum coreanum,* using lipopolysaccharide (LPS)/interferon-gamma (IFN-γ)-induced RAW264.7 macrophage cells, on anti-inflammation were investigated. Our study was focused on the ethyl acetate fraction from *T. coreanum* (ETC) and its active compounds and their protective role against inflammation. The ETC and its active compounds, LT and LS, showed dose-dependent inhibitory activity against the production of nitric oxide (NO) and reactive oxygen species (ROS) in LPS/IFN-γ-stimulated RAW264.7 cells. In addition, ETC and its active compounds inactivated nuclear factor-kappa B and down-regulated inflammatory mediators. The results also showed that treatment with ETC, LT, and LS decrease pro-inflammatory cytokines, tumor necrosis factor-alpha, and interleukin-6. In conclusion, our studies indicated that ETC has anti-inflammatory activity owing to inhibition of NO/ROS generation and down-regulation of inflammatory mediators and cytokines. Moreover, LT and LS are bioactive compounds of ETC with protective effects against inflammation.

Keywords Anti-inflammatory activity · Luteolin · Luteoloside · RAW264.7 macrophage cell · *Taraxacum coreanum*

✉ Sanghyun Lee
slee@cau.ac.kr

✉ Eun Ju Cho
ejcho@pusan.ac.kr

[1] Department of Food Science and Nutrition & Research Institute of Ecology for the Elderly, Pusan National University, Busan 46241, Republic of Korea

[2] Natural Products Research Center, KIST Gangneung Institute, Gangneung 25451, Republic of Korea

[3] Department of Food Science, Gyeongnam National University of Science and Technology, Jinju 52725, Republic of Korea

[4] Department of Integrative Plant Science, Chung-Ang University, Anseong 17546, Republic of Korea

Introduction

Inflammation is the biological response against pathogens that cause cell injury in the human body. Normal inflammation is immune response against harmful stimuli or damage by up-regulation of anti-inflammatory cytokines/mediators (Lawrence et al. 2002). However, chronic inflammation is associated with pro-inflammatory cytokines production and increased risk of tissue damage and other degenerative disorders (Allavena et al. 2008). The stimulation of macrophages with lipopolysaccharide (LPS) leads to the secretion of inflammatory cytokines. Nuclear factor-kappa B (NF-κB) is activated by inflammatory stimuli, such as LPS; activated NF-κB modulates the expressions of pro-inflammatory enzymes and cytokines (Karin and Ben-Neriah 2000). Nitric oxide (NO) is a reactive radical which is produced by inducible nitric oxide synthase (iNOS), and regulates physiological and pathological conditions (Sacco et al. 2006). However, the over-expression of NO induces various harmful responses such as tissue damage and acute or chronic inflammatory diseases (Kaplanski et al. 2003). Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) can induce inflammatory progression through mediators, iNOS and cyclooxygenase-2 (COX-2) (Warren 1990). NF-κB-targeted treatments might be effective against diseases,

because of its role in the pathogenesis of inflammatory gene expression.

Taraxacum coreanum (TC) is a plant native to Korea and Japan. TC is used as a medicine for the treatment of diuretic disorder or inflammation (Koo et al. 2004; Lee et al. 2013). Recent studies also demonstrated that it has hypolipidemic and anti-oxidant activity against oxidative stress (Chiou et al. 1997). Flavonoids are polyphenol compounds that are important to human health. They possess a wide range of biological effects on inflammation, allergy, and asthma (Middleton and Kandaswami 1992). Wolbis et al. (1993) identified that flavonoids, quercetin, luteolin (LT), luteolin-7-glucoside (LS), and quercetin-7-glucoside in dandelion leaves and flower extracts. In particular, the total content of LT and LS in TC was contained more than that in *T. ohwianum* and *T. officinale* (Lee et al. 2011). However, the anti-inflammatory activity of the active compounds in TC has not been studied yet.

To investigate the anti-inflammatory effects of ethyl acetate (EtOAc) fraction of TC (ETC) and its active compounds, we used LPS- and interferon-gamma (IFN-γ)-stimulated RAW264.7 cells to induce inflammation by increased NO production. In addition, the regulatory mechanisms against the inflammatory process were also studied by measurement of the levels of inflammatory cytokines and mediators induced by NF-κB activation in LPS/IFN-γ-stimulated RAW264.7 cells.

Materials and methods

Plant materials

Aerial parts of TC collected at sides of the West Coast Express Highway (geographic coordinates: 36°53′35″N 126°37′41″E) in the Republic of Korea by permission of Korea Expressway Corporation were used.

Instruments and reagents

RAW264.7 macrophage cells from Korea Cell Line Bank (KCLB, Seoul, Korea) were used. To culture cells, fetal bovine serum (FBS), Dulbecco's modified eagle's medium (DMEM), and penicillin/streptomycin were supplied from Welgene (Daegu, Korea). IFN-γ was from Pepro Tech (Rocky Hill, NJ, USA). The LPS, Griess reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were supplied from Sigma Chemical Co. (St Louis, MO, USA).

Preparation of ETC and its active compounds

Freeze-dried TC was extracted with methanol (MeOH) for 3 h; the process of MeOH extraction was repeated 8 times. The extract was concentrated by a rotary evaporator and suspended in water. The combined extract was partitioned with *n*-hexane, chloroform ($CHCl_3$), EtOAc, and *n*-butanol (*n*-BuOH), successively. A portion of the EtOAc fraction was subjected to a silica gel column chromatography using a gradient system of *n*-hexane–EtOAc and EtOAc–MeOH to yield LT and LS (Fig. 1).

Cell culture

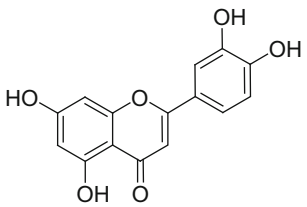
The RAW264.7 cells maintained at 37 °C in a CO_2 (5 %) incubator with DMEM containing penicillin/streptomycin (1 %) and FBS (10 %) were sub-cultured weekly with 0.05 % trypsin-ethylenediaminetetraacetic acid (EDTA) in PBS.

Cell viability

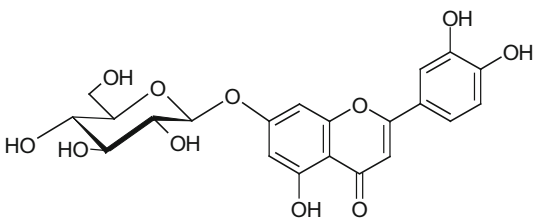
After the cells reached confluence, the cells were seeded at 5×10^4 cells/well into 24-well plates, for 2 h incubation, and then treated with samples for 24 h. RAW264.7 cells were then stimulated with LPS (1 μg/mL)/IFN-γ (10 ng/mL) for 24 h. The cells were incubated with 1 mL of MTT solution (5 mg/mL) for 4 h at 37 °C, and the medium containing MTT was removed. And then, the formazan crystals were dissolved with 1 mL of DMSO, and viable cells were detected to measure absorbance at 540 nm (Mosmann 1983).

Measurement of NO production

The NO production was investigated as the nitrite accumulation in the medium treated with the Griess reagent. To



LT



LS

Fig. 1 Chemical structures of LT and LS

measure nitrite, the cell supernatants were added with a same volume of the Griess reagent. And then, the nitrite concentration was measured using microplate spectrophotometer at a wave length of 540 nm.

Measurement of ROS production

The ROS scavenging activity of ETC and its active compounds was measured using dichlorodihydrofluorescein diacetate (DCFH-DA) (Cathcart et al. 1983). RAW264.7 cells were incubated with LPS/IFN-γ for 24 h. After that, ETC (5, 25, 50, and 100 μg/mL) and its active compounds, LT and LS (0.5, 2.5, 5, and 10 μg/mL), were added for 24 h at 37 °C. Then florescence was read for 60 min, at wavelengths of 480 nm for excitation and 535 nm for emission, using a florescence plate reader (BMG LABTECH, Ortenberg, Germany).

RNA extraction and reverse transcription polymerase chain reaction (RT-PCR)

According to the manufacturer's instruction, total RNA was isolated using a Trizol reagent (Invitrogen, Carlsbad, CA, USA). Cells were lysed by the Trizol reagent and RT-PCR was performed using TOP script One-step RT-PCR (Enzynomics, Daejeon, Korea). The RNA was reverse-transcribed into cDNA which is used as a template for amplification of RT-PCR (Table 1). The products of PCR were analyzed on 1 % agarose gels; the expression was visualized under LED slider imager (Maestrogen, NV, USA).

Statistical analysis

Data are expressed as the mean ± SD. Statistical significance was measured by one-way ANOVA, followed by Duncan's post hoc tests ($P < 0.05$).

Results

Effect of ETC and its active compounds on NO production

LPS/IFN-γ treatments induced NO formation in RAW264.7 cells (Fig. 2). Exposure of LPS/IFN-γ-stimulated RAW264.7 cells to ETC (5, 25, 50, and 100 μg/mL) and its active compounds, LT and LS (0.5, 2.5, 5, and 10 μg/mL), for 24 h did not have any cytotoxic effects at a concentration lower than 100 μg/mL ETC and 10 μg/mL active compounds (data not shown). LPS significantly increased NO levels, whereas the treatment with various concentrations of ETC remarkably inhibited NO production in LPS/IFN-γ-activated RAW264.7 cells dose-dependently. In addition, we checked the effects of LT and LS on NO production. The results showed that NO production was decreased by LT and LS treatments, in a dose-dependent manner. Especially, treatments with LT at 2.5, 5, and 10 μg/mL suppressed NO production by 46.07, 43.98, and 42.18 %, respectively, compared to LPS/IFN-γ-stimulated control group (100 %). These results suggested that both ETC and its active compounds have inhibitory effects on NO production in LPS/IFN-γ-stimulated RAW264.7 macrophage cells.

Effect of ETC and its active compounds on ROS production

Exposure of RAW 264.7 cells to LPS/IFN-γ for 24 h increased intracellular ROS levels (Fig. 3). In contrast, ETC treatments prevented the increase of LPS/IFN-γ-induced ROS production. When the cells were treated with ETC at 100 μg/mL, ROS production decreased by 71.27 % compared to the control group (100 %). In addition, the active compounds markedly decreased ROS formation in the presence of LPS/IFN-γ (Fig. 3). In particular, LT had a stronger ROS scavenging activity than LS.

Table 1 Primers and conditions used in PCR

mRNA	Primer sequence	PCR conditions
NF-κB	F: GCA-GCC-TAT-CAC-CAA-CTC-T	48 °C
	R: TAC-TCC-TTC–TTC-ACC-A	Cycle: 35
iNOS	F: CCT–CCT-CCA-CCC-TAC-CAA-GT	53 °C
	R: CAC-CCA-AAG-TGC-CTC-AGT-CA	Cycle: 35
COX-2	F: AAG-ACT-TGC-CAG-GCT-GAA-CT	53 °C
	R: CTT-CTG-CAG-TCC-AGG-TTC-AA	Cycle: 35
GAPDH	F: TCA-TGA-AGT-GTG-ACG-TTG-ACA-TCC-GT	60 °C
	R: CCT-AGA-AGC-ATT-TGC-GGT-GCA-CGA-TG	Cycle: 35

NF-κB Nuclear factor-kappa B, *iNOS* inducible nitric oxide synthase, *COX-2* cyclooxygenase-2, *GAPDH* Glyceraldehyde 3-phosphate dehydrogenase

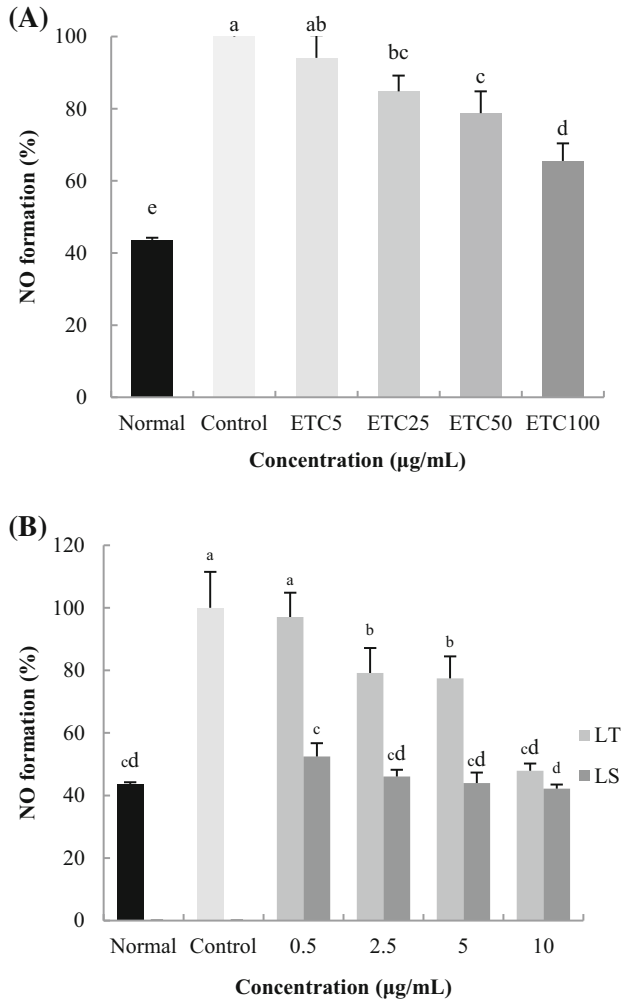


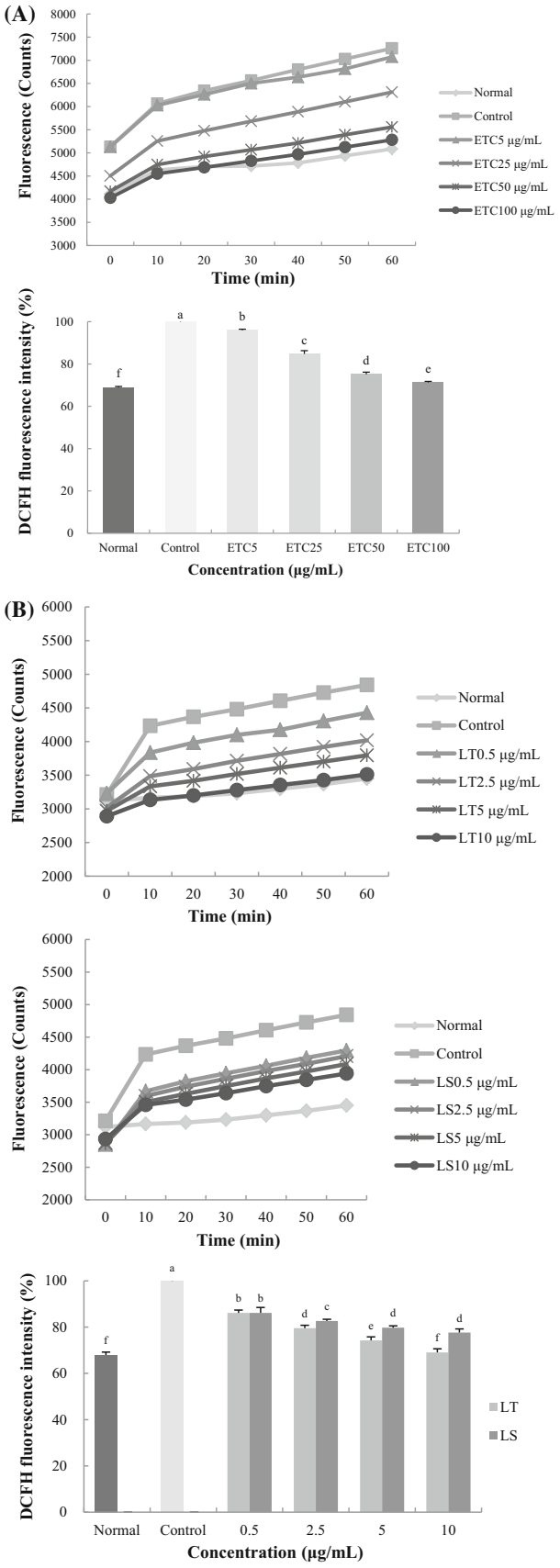
Fig. 2 Effects of ETC **(A)**, LT, and LS **(B)** on NO production in RAW264.7 cells treated with LPS/IFN-γ. Values are the mean ± SD. *a–e* Means with different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test

Effect of ETC and its active compounds on TNF-α and IL-6 release

Figure 4 shows the effect of ETC and its active compounds on production of TNF-α and IL-6 in LPS/IFN-γ-stimulated RAW264.7 cells. As a result, treatments with ETC and its active compounds inhibited the production of TNF-α and IL-6 dose-dependently. LT inhibited TNF-α and IL-6 production more significantly than LS, suggesting that LT inhibited TNF-α and IL-6 production more significantly than its glycoside LS in LPS/IFN-γ-stimulated RAW264.7 cells.

Effect of ETC and its active compounds on mRNA expression of NF-κB, iNOS, and COX-2

As shown in Fig. 5, LPS/IFN-γ significantly increased mRNA expressions of pro-inflammatory mediators, NF-



◀**Fig. 3** Effect of ETC **(A)**, LT, and LS **(B)** on levels of ROS in RAW264.7 cells treated with LPS/IFN-γ. Values are the mean ± SD. *a–f* Means with different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test

κB, iNOS, and COX-2, in the control group compared to untreated group. However, treatments with ETC and its active compounds, LT and LS, suppressed NF-κB, iNOS, and COX-2 expressions dose-dependently. These results indicated that treatments with ETC and its active compounds block the degradation of NF-κB activation, and therefore, iNOS and COX-2 expressions were attenuated in LPS/IFN-γ-stimulated RAW264.7 cells.

Discussion

Chronic inflammatory diseases were accompanied by increased ROS production and inflammatory gene expressions (Wang et al. 1994; Wiseman and Halliwell 1996). TC, a member of Asteraceae, has been used as a medicinal herb to treat diuretic and liver disease. Several reports suggested that flavonoids, one of the large families of plant constituents, have inhibitory activity against inflammation (Havsteen 1983; Kim et al. 2004). According to Hu and Kitts, LT and LS, which are flavones isolated from dandelion flower extract, alleviated oxidative reaction (Hu and Kitts 2003). In our previous study, the EtOAc fraction had the strongest anti-oxidant property compared to the other fractions (Lee et al. 2012). Therefore, we focused on the anti-inflammatory effects of ETC (5, 25, 50, and 100 μg/mL) and the active compounds (1, 2.5, 5, and 10 μg/mL) isolated from ETC. The concentration of active compounds, LT and LS (1, 2.5, 5, and 10 μg/mL), is equivalent to the 1.7, 8.7, 17.5, and 35 μM of LT and 1.1, 5.6, 11.1, and 22.3 μM of LS, respectively. We hypothesized that ETC and its active compounds regulate ROS generation and inflammatory gene expression.

Macrophage cells are important to inflammation reaction induced by cytotoxicity and multiple inflammatory diseases through cellular response (MacMiking et al. 1997). RAW 264.7 macrophage cells stimulated by LPS/IFN-γ enhanced the expressions of inflammatory cytokines and mediators (Xie and Nathan 1994). The over-production of ROS by macrophage cells is one of the most important hallmarks of inflammatory reaction. D'Acquisto et al. (2002) reported that ROS are involved in cellular stress and modulation of NF-κB activation. Therefore, inhibition of ROS production is considered a therapeutic target for preventing inflammatory diseases. ETC, LT, and LS showed the most potent anti-oxidant and anti-inflammatory effects through inhibition of ROS generation (Fig. 3). These results suggested that the anti-oxidative and anti-inflammatory activities of ETC are attributed to its active compounds, LT and LS.

NO, a reactive free radical, is produced by L-arginine and is central role in the inflammatory process or infection (Marletta 1993). At adequate concentrations, NO can generate and regulate intracellular signals, thereby affecting the immune cells. However, uncontrolled production of NO is associated with inflammation. Our results demonstrated that ETC and its active compounds have concentration-dependent inhibition of NO production. Moreover, treatments with LT and LS on RAW264.7 cells stimulated with LPS/IFN-γ significantly reduced NO production. The previous reports demonstrated that cytotoxicity of LS was lower than its aglycone form, LT. Other reports confirmed that glycosylation may contribute to reduced toxicity and that LS had stronger NO suppression activity than LT (Wang and Mazza 2002a; Hu and Kitts 2004).

To investigate the inhibitory mechanisms of NO production, the effects of ETC and its active compounds on mRNA expressions in LPS/IFN-γ-induced RAW264.7 cells were determined. NF-κB is a transcriptional activation factor that plays a crucial role in the inflammatory response, including the regulation of iNOS and COX-2 (Spitzer et al. 2002). Therefore, iNOS and COX-2 expressions, and NF-κB activation have been used as biomarkers for anti-inflammatory activity. The present results indicated that ETC, LT, and LS suppressed NF-κB activation as well as LPS/IFN-γ-induced iNOS and COX-2 mRNA expression. Previous study mentioned that phenolic compounds inhibited inflammatory mediators and suppressed inflammatory responses through NF-κB pathway (Rao et al. 2005; Jung et al. 2007). Therefore, it is strongly suggested that the inhibition of NO production may be owing to decreased iNOS and COX-2 expressions.

TNF-α and IL-6 are multifunctional inflammatory cytokines. These cytokines contribute to tissue damage and multiple organ failure (Akira et al. 1990; Hirano 1992). IL-6 is a pro-inflammatory cytokine involved in the regulation of immune responses, which are induced by macrophages (Tilg et al. 1994). Moreover, a large amount of TNF-α secretion produced by LPS leads to NO generation (Aggarwal and Natarajan 1996). Thus, the inhibition of cytokine production is important for potential anti-inflammatory activity. Several studies demonstrated that phenolic compounds possessed TNF-α and IL-6 inhibitory activities (Xagorari et al. 2001; Wang and Mazza 2002b). In addition, dandelion leaf extract suppressed the TNF-α production by inhibiting IL-1 production in LPS-stimulated rat astrocytes (Kim et al. 2000). These results indicated that the production of TNF-α and IL-6 significantly increased in LPS/IFN-γ-stimulated RAW264.7 cells. However, ETC, LT, and LS possess inhibitory efficacy against the

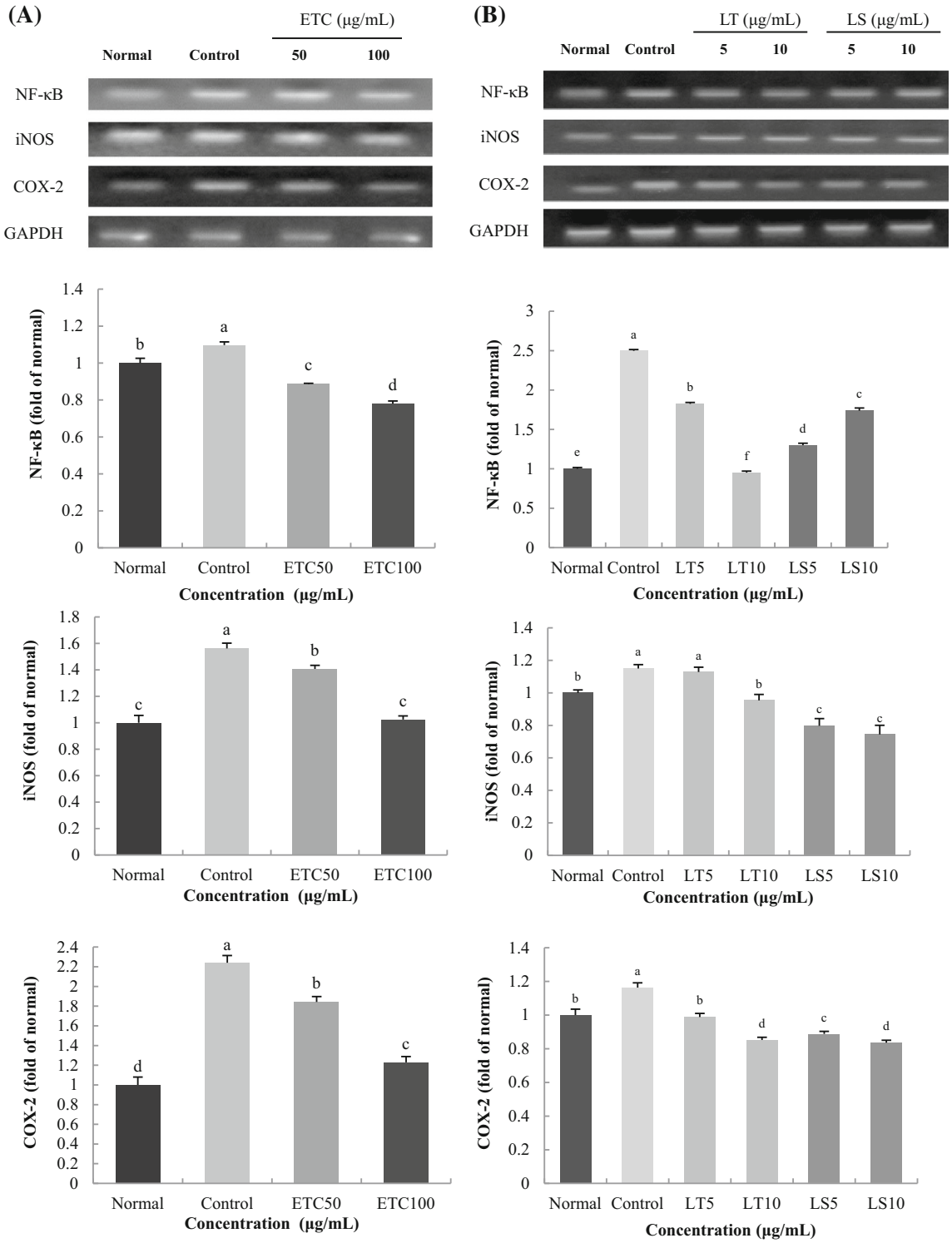
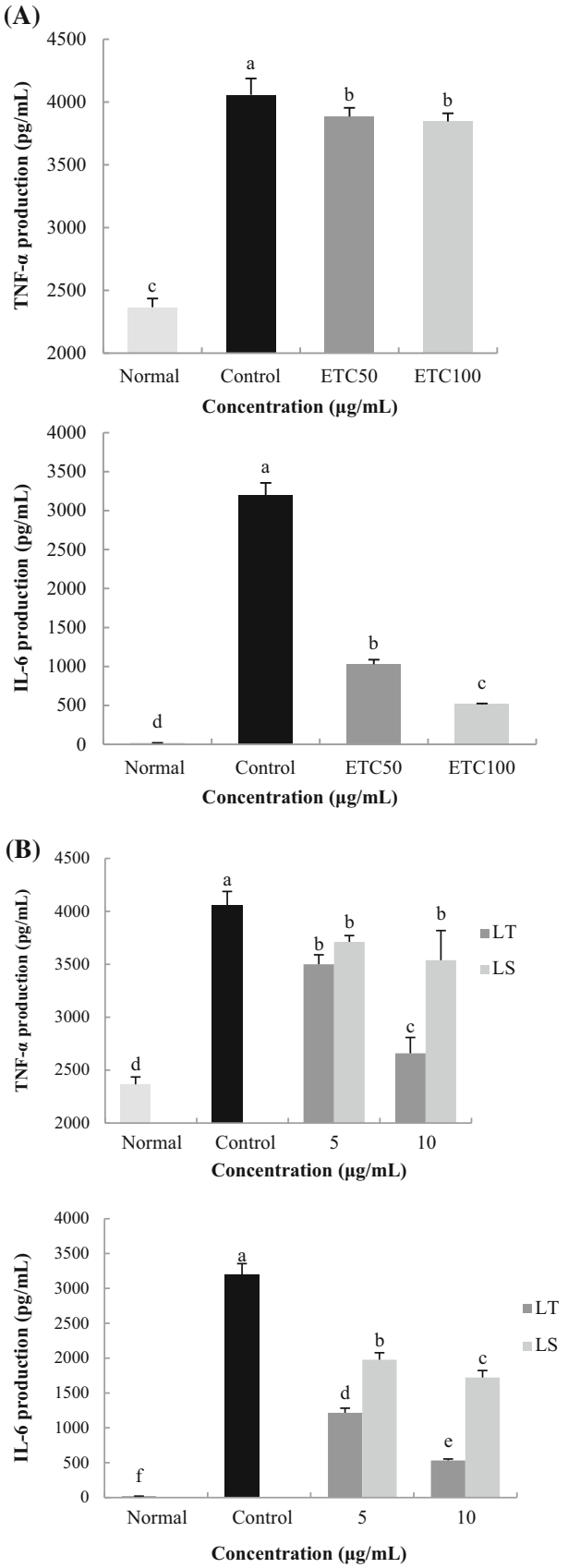


Fig. 4 Effect of ETC **(A)**, LT, and LS **(B)** on mRNA expressions of NF-κB, iNOS, and COX-2 in RAW264.7 cells treated with LPS/IFN-γ. Values are the mean ± SD. *a–f* Means with different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test

production of TNF-α and IL-6. In particular, ETC and its active compounds showed stronger inhibitory effects against IL-6 than TNF-α production. These results suggested that down-regulation of IL-6 might be related to its production of TNF-α. In addition, compared to the aglycone form, the glycoside form had stronger inhibitory effects against TNF-α and IL-6 production in RAW264.7 macrophage cells.



◀**Fig. 5** Effect of ETC, LT, and LS on TNF-α **(A)** and IL-6 production **(B)** in RAW264.7 cells treated with LPS/IFN-γ. Values are the mean ± SD. *a–e* Means with different letters are significantly different ($P < 0.05$) as determined by Duncan's multiple range test

To investigate the anti-inflammatory effect of LT aglycone and its glycosides, the production of NO and cytokines, which have crucial role during the inflammatory process by defensing against foreign agents, was measured. Our study showed that LT and LS significantly inhibited production of NO dose-dependently. In particular, LS had a strong NO scavenging effect rather than LT by down-regulating iNOS and COX-2 gene expression. In contrast to inhibitory effect of LS on NO generation, treatment of LT resulted in a more considerable decrease in TNF-α and IL-6 secretion in LPS/IFN-γ-stimulated RAW264.7 macrophage cells. Previous study has also reported that stronger suppression of NO production was observed in LS-treated group than LT-treated group (Hu and kitts 2004). However, Park and Song (2013) found that LT was more effective in inhibition of LPS-induced prostaglandin E_2 (PGE_2) production and up-regulation of NF-κB and activator protein (AP)-1 expression. These findings suggest that LT and LS display a selective activity in secretion of pro-inflammatory cytokines, NO production, and regulation of inflammation-related mRNA expression. Taken together, the inhibition of NO production by LS was attributed to the suppression of iNOS and COX-2 expression, rather than a cytokines. On the other hand, LT plays anti-inflammatory role by suppression of TNF-α and IL-6 secretion.

In conclusion, the present study revealed that ETC and its active compounds, LT and LS, had anti-inflammatory effects on LPS/IFN-γ-induced RAW264.7 cells. They inhibited the generation of ROS and NO induced by LPS/IFN-γ. In addition, activation of NF-κB was inhibited by the expressions of iNOS and COX-2, and the production of TNF-α and IL-6 was inhibited by the treatment with LT and LS. These results suggest that ETC would play a beneficial role in alleviating the inflammatory process and that LS and LT are attributed to the protective role of ETC.

References

Aggarwal BB, Natarajan K (1996) Tumor necrosis factors: developments during the last decade. Eur Cytokine Netw 7:93–124

Akira S, Hirano T, Taga T, Kishimoto T (1990) Biology of multifunctional cytokines: IL-6 and related molecules (IL-1 and TNF). FASEB J 4:2860–2867

Allavena P, Garlanda C, Borrello MG, Sica A, Mantovani A (2008) Pathways connecting inflammation and cancer. Curr Opin Genet Dev 18:3–10

Cathcart R, Schwiers E, Ames BN (1983) Detection of picomole levels of hydroperoxides using a fluorescent dichlorofluorescein assay. Anal Biochem 134:111–116

Chiou WF, Sung YJ, Liao JF, Shum AY, Chen CF (1997) Inhibitory effect of dehydroevodiamine and evodiamine on nitric oxide production in cultured murine macrophages. J Nat Prod 60:708–711

D'Acquisto F, May MJ, Ghosh S (2002) Inhibition of nuclear factor kappa B (NF-B): an emerging theme in anti-inflammatory therapies. Mol Intervent 2:22–35

Havsteen B (1983) Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharmacol 32:1141–1148

Hirano T (1992) Interleukin-6 and its relation to inflammation and disease. Clin Immunol Immunopathol 62:560–565

Hu C, Kitts DD (2003) Antioxidant, prooxidant and cytotoxic activities of solvent fractionated dandelion (*Taraxacum officinale*) flower extracts in vitro. J Agric Food Chem 52:301–310

Hu C, Kitts DD (2004) Luteolin and luteolin-7-*O*-glucoside from dandelion flower suppress iNOS and COX-2 in RAW 264.7 cells. Mol Cell Biochem 265:107–113

Jung CH, Kim JH, Hong MH, Seog HM, Oh SH, Lee PJ, Kim GJ, Kim HM, Um JY, Ko SG (2007) Phenolic-rich fraction from *Rhus verniciflua* Stokes (RVS) suppress inflammatory response via NF-κB and JNK pathway in lipopolysaccharide-induced RAW 264.7 macrophages. J Ethnopharmacol 110:490–497

Kaplanski G, Marin V, Montero-Julian F, Mantovani A, Farnarier C (2003) IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation. Trends Immunol 24:25–29

Karin M, Ben-Neriah Y (2000) Phosphorylation meets ubiquitination: the control of NF-κB activity. Ann Rev Immunol 18:621–663

Kim HM, Shin HY, Lim KH, Ryu ST, Shin TY, Chae HJ, Kim HR, Lyu YS, An NH, Lim KS (2000) *Taraxacum officinale* inhibits tumor necrosis factor-α production from rat astrocytes. Immunopharmacol Immunotoxicol 22:519–530

Kim HP, Son KH, Chang HW, Kang SS (2004) Anti-inflammatory plant flavonoids and cellular action mechanisms. J Pharmacol Sci 96:229–245

Koo HN, Hong SH, Song BK, Kim CH, Yoo YH, Kim HM (2004) *Taraxacum officinale* induces cytotoxicity through TNF-α and IL-1α secretion in Hep G2 cells. Life Sci 74:1149–1157

Lawrence T, Willoughby DA, Gilroy DW (2002) Anti-inflammatory lipid mediators and insights into the resolution of inflammation. Nat Rev Immunol 2:787–795

Lee S, Han S, Kim HM, Lee JM, Mok SY, Lee S (2011) Isolation and identification of phytochemical constituents from *Taraxacum coreanum*. J Korean Soc Appl Biol Chem 54:73–78

Lee S, Choi MJ, Choi JM, Lee S, Kim HY, Cho EJ (2012) Flavonoids from *Taraxacum coreanum* protect from radical-induced oxidative damage. J Med Plants Res 6:5377–5384

Lee MH, Kang H, Lee K, Yang G, Ham I, Bu Y, Kim H, Choi HY (2013) The aerial part of *Taraxacum coreanum* extract has an anti-inflammatory effect on peritoneal macrophages in vitro and increases survival in a mouse model of septic shock. J Ethnopharmacol 146:1–8

MacMiking J, Xie Q, Nathan C (1997) Nitric oxide and macrophage function. Ann Rev Immunol 15:323–350

Marletta MA (1993) Nitric oxide synthase structure and mechanism. J Biol Chem 268:12231–12234

Middleton E, Kandaswami C (1992) Effects of flavonoids on immune and inflammatory cell functions. Biochem Pharmacol 43: 1167–1179

Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65:55–63

Park CM, Song YS (2013) Luteolin and luteolin-7-*O*-glucoside inhibit lipopolysaccharide-induced inflammatory responses through modulation of NF-κB/AP-1/PI3 K-Akt signaling cascades in RAW 264.7 cells. Nutr Res Pract 7:423–429

Rao YK, Fang SH, Tzeng YM (2005) Inhibitory effects of the flavonoids isolated from *Waltheria indica* on the production of NO, TNF-α and IL-12 in activated macrophages. Biol Pharm Bull 28:912–915

Sacco RE, Waters WR, Rudolph KM, Drew ML (2006) Comparative nitric oxide production by LPS-stimulated monocyte-derived macrophages from *Ovis canadensis* and *Ovis aries*. Comp Immunol Microbiol Infect Dis 29:1–11

Spitzer JA, Zheng M, Kolls JK, Stouwe CV, Spitzer JJ (2002) Ethanol and LPS modulate NF-kappaB activation, inducible NO synthase and COX-2 gene expression in rat liver cells in vivo. Front Biosci 7:99–108

Tilg H, Trehu E, Atkins MB, Dinarello CA, Mier JW (1994) Interleukin-6 (IL-6) as an anti-inflammatory cytokine: induction of circulating IL-1 receptor antagonist and soluble tumor necrosis factor receptor p55. Blood J 83:113–118

Wang J, Mazza G (2002a) Inhibitory effects of anthocyanins and other phenolic compounds on nitric oxide production in LPS/IFN-γ-activated RAW 264.7 macrophage. J Agric Food Chem 50:850–857

Wang J, Mazza G (2002b) Effects of anthocyanins and other phenolic compounds on the production of tumor necrosis factor α in LPS/IFN-γ-activated RAW 264.7 macrophages. J Agric Food Chem 50:4183–4189

Wang SC, Rossignol DP, Christ WJ, Geller DA, Freeswick PD, Thai NL, Su GL, Simmons RL (1994) Suppression of lipopolysaccharide-induced macrophage nitric oxide and cytokine production in vitro by a novel lipopolysaccharide antagonist. Surg J 116:339–346

Warren JS (1990) Interleukins and tumor necrosis factor in inflammation. Crit Rev Clin Lab Sci 28:37–59

Wiseman H, Halliwell B (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. Biochem J 313:17–29

Wolbis M, Kolikowska M, Bednarek P (1993) Polyphenolic compounds in *Taraxacum officinale*. Acta Poloniae Pharm 50: 153–158

Xagorari A, Papapetropoulos A, Mauromatis A, Economou M, Fotsis T, Roussos C (2001) Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and proinflammatory cytokine production in macrophages. J Pharmacol Exp Ther 296:181–187

Xie Q, Nathan C (1994) The high-output nitric oxide pathway: role and regulation. J Leukoc Biol 56:576–582