



Sesamol suppresses the inflammatory response by inhibiting NF- κ B/MAPK activation and upregulating AMP kinase signaling in RAW 264.7 macrophages

Xin-Ling Wu¹ · Chian-Jiun Liou² · Zih-Ying Li¹ · Xuan-Yu Lai¹ · Li-Wen Fang³ · Wen-Chung Huang^{4,5}

Received: 17 March 2015 / Revised: 12 May 2015 / Accepted: 29 May 2015 / Published online: 10 June 2015
© Springer Basel 2015

Abstract

Objectives and design Sesamol is a lignan isolated from sesame seed oil. In recent years, it was found that sesamol could decrease lung inflammation and lipopolysaccharide (LPS)-induced lung injury in rats. In this study, we investigated whether sesamol exhibited anti-inflammatory activity in LPS-stimulated macrophages.

Materials and methods RAW 264.7 cells were treated with sesamol, then treated with LPS to induce inflammation. The levels of proinflammatory cytokines were analyzed with ELISA. The gene and protein expression of cyclooxygenase (COX)-2, inducible nitric oxide synthase (iNOS), and nuclear factor erythroid-2-related factor 2 (Nrf2) were evaluated with real-time PCR and Western

blots, respectively. We also examined inflammatory signaling pathways, including nuclear transcription factor kappa-B (NF- κ B) and mitogen-activated protein kinase (MAPK) pathways.

Results Sesamol inhibited production of nitric oxide, prostaglandin E₂ (PGE₂), and proinflammatory cytokines. Sesamol markedly suppressed mRNA and protein expression of iNOS and COX-2. Sesamol enhanced the protective antioxidant pathway represented by Nrf2 and HO-1. Moreover, sesamol suppressed NF- κ B transport into the nucleus and decreased MAPK activation, but it promoted adenosine monophosphate-activated protein kinase (AMPK) activation.

Conclusions These data suggested that sesamol ameliorated inflammatory and oxidative damage by upregulating AMPK activation and Nrf2 signaling and blocking the NF- κ B and MAPK signaling pathways.

Responsible Editor: John Di Battista.

X.-L. Wu and C.-J. Liou have contributed equally for the paper.

✉ Wen-Chung Huang
wchuang@gw.cgust.edu.tw

- ¹ Department of Nutrition and Health Sciences, Chang Gung University of Science and Technology, 261 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan, Taiwan
- ² Department of Nursing, Chang Gung University of Science and Technology, 261 Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan, Taiwan
- ³ Department of Nutrition, I-Shou University, No. 8, Yida Rd., Jiaosu Village, Yanchao District, Kaohsiung 82445, Taiwan
- ⁴ Graduate Institute of Health Industry Technology, Chang Gung University of Science and Technology, No. 261, Wenhua 1st Rd., Kwei-Shan, Guishan Dist., Tao-Yuan 33303, Taiwan
- ⁵ Research Center for Industry of Human Ecology, Chang Gung University of Science and Technology, Kwei-Shan, Tao-Yuan, Taiwan

Keywords AMPK · COX-2 · HO-1 · MAPK · NF- κ B · Sesamol

Introduction

Inflammation is an important warning sign for acute and chronic diseases, including bacterial infections, acute lung injury, obesity, and cancers [1, 2]. Controlling the inflammatory response was determined to be an important treatment strategy [3]. Lipopolysaccharide (LPS) is the primary component of the cell walls of gram-negative bacteria, and it can trigger an inflammatory response and stimulate the activity of immune cells against a bacterial invasion [4]. LPS binds to the TLR 4 receptor of macrophages to stimulate proinflammatory cytokine and mediator production [5]. However, excessive inflammatory

responses can cause physical discomfort and may lead to fever, hypotension, shock, severe sepsis, and death [4].

Proinflammatory cytokines and inflammatory mediators are involved in physiological responses to chronic and acute inflammation. For example, nuclear transcription factor kappa-B (NF- κ B) is transported into the nucleus to activate inflammatory gene expression [6]. In the inactive state, NF- κ B is a heterodimer that consists of p50 and p65 proteins. It is constitutively localized in the cytoplasm, and its movement is restrained by the inhibitor of nuclear factor kappa B (I κ B) [7]. When gram-negative bacteria infect the cell, LPS stimulates the activation of macrophages. This activation induces the phosphorylation of I κ B, which releases NF- κ B to move to the nucleus and stimulate the expression of proinflammatory cytokines, COX-2 and iNOS. The mitogen-activated protein kinase (MAPK) signaling pathway also plays an important role in up-regulating inflammatory gene expression in LPS-stimulated macrophages [8]. Several studies found that phosphorylation of MAPKs could induce NF- κ B activation and regulate inflammatory gene expression [7, 9]. Hence, suppressing NF- κ B and MAPK pathways could ameliorate the inflammatory response.

Hemeoxygenase-1 (HO-1) maintains cellular redox homeostasis and protects against oxidative stress [10]. Inflammatory cytokines, LPS, and oxidative stress can induce HO-1 expression [1] by activating nuclear factor erythroid-2-related factor (Nrf2). Nrf2 is a transcription factor that translocates into the nucleus and binds to the antioxidant responsive element, which controls the expression of HO-1. The HO-1 protein acts against inflammatory responses and protects the cell from oxidative damage [11]. Adenosine monophosphate-activated protein kinase (AMPK) is an important factor that regulates energy balance [12]. Recent studies found that, in addition to its beneficial effect on metabolic disorders, AMPK activation also decreased inflammatory responses in patients with metabolic syndromes [13, 14].

A recent study discovered that some natural products could ameliorate antioxidant responses and the inflammatory response in LPS-stimulated macrophages [9, 15, 16]. *Sesamun indicum*, Linn. (Sesame) is an important oil crop [17]. Sesame seed oil contains high levels of unsaturated fatty acids, including linoleic acid and linolenic acid [18]. Sesame seed oil also contains beneficial vitamins and minerals that regulate physiological functions [19]. Sesamol (3,4-methylenedioxyphenol) is a polyphenol lignan isolated from sesame seeds [20]. Studies found that sesamol could induce apoptosis in HepG2 cells [21]. Sesamol also reduced cholesterol and triacylglycerol levels in hyperlipidemic mice [22]. In addition, sesamol was reported to have an anti-inflammatory effect in a rat inflammatory lung model and an antioxidant effect in a rat

acute pancreatitis model [23]. However, the molecular mechanism is unclear for its anti-inflammatory effects. Therefore, in this study, we evaluated the anti-inflammatory effects of sesamol, and we investigated whether its mechanism is involved in the NF- κ B and MAPK signaling pathways in LPS-induced RAW 264.7 macrophage cells.

Materials and methods

Materials

The chemical structure of sesamol (from *Sesamun indicum*, ≥ 98 % purity by HPLC, Sigma-Aldrich Co, St. Louis, MO, USA) is illustrated in Fig. 1a. Sesamol was dissolved in phosphate-buffered saline.

Cell culture

The RAW 264.7 murine macrophage cell line was obtained from the Bioresource Collection and Research Center (BCRC, Taiwan). Cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies, Carlsbad, CA, USA) with 10 % fetal bovine serum (Biological Industries, Haemek, Israel), 2 mM L-glutamine, 100 units/ml penicillin, and 100 μ g/ml streptomycin. All cells were incubated at 37 °C in 5 % CO₂ humidified air, and cells were subcultured twice each week.

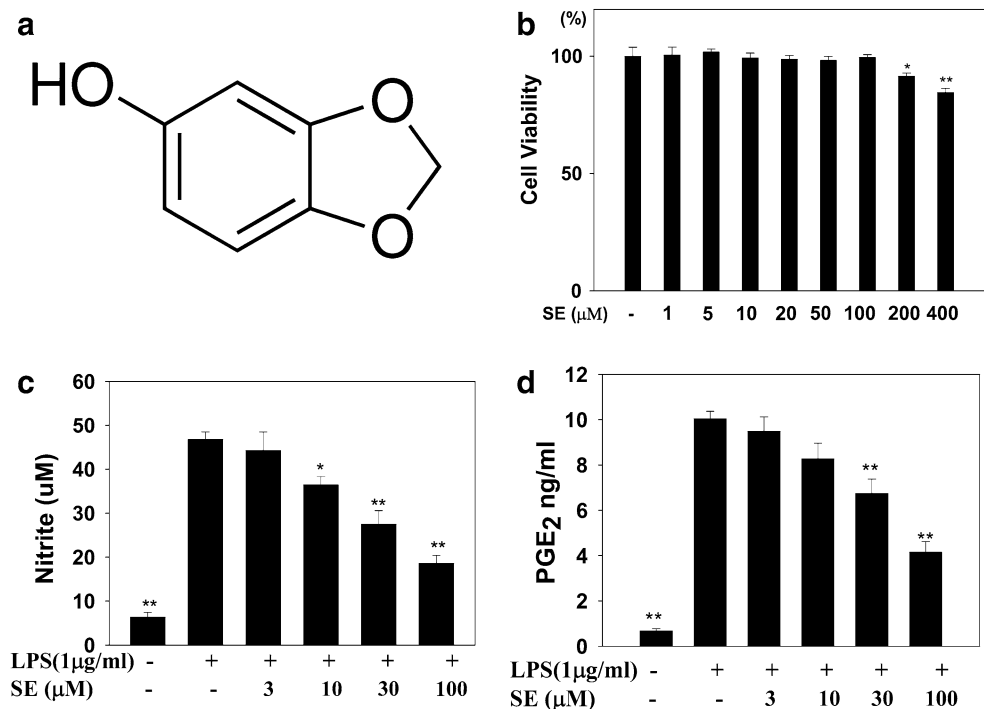
Cell viability assay

The effects of sesamol on cell viability were determined with the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, as previously described [9]. In brief, the cells (5×10^3 cells/well) were seeded in 96-well plates and treated with sesamol for 24 h. Then, cells were treated with 5 mg/ml MTT solution for 4 h at 37 °C. Next, the plate was washed and, after adding formazone crystals dissolved in isopropanol, we measured the absorbance of the resulting color at OD570 nm with a multi-detection reader (Multiskan FC, Thermo, Waltham, MA, USA).

Determination of nitric oxide (NO) production

The cells were seeded (5×10^5 cells/well) in 24-well plates and treated with sesamol. Then, cells were treated with 1 μ g/ml LPS (*Escherichia coli* serotype 026:B6, Sigma) for 24 h. Nitrite in the culture medium was measured with Griess reagent (Sigma) as an indicator of nitric oxide production, as previously described [16]. The absorbance at 570 nm was measured with a multi-detection reader (Multiskan FC, Thermo).

Fig. 1 Properties of sesamol.
a Chemical structure.
b Cytotoxicity. RAW264.7 cell viability was tested with the MTT assay in the presence of the indicated concentrations of sesamol (SE). Effects of sesamol on LPS-induced production of (c) nitrite and (d) PGE₂. Here, cells (5×10^5 cells/well) were seeded in 24-well plate, pretreated with sesamol (SE) for 1 h, and stimulated with LPS (1 μ g/ml) for 24 h. The data represent the mean \pm SD; * $p < 0.05$, ** $p < 0.01$, compared to LPS alone



Measurements of cytokines, chemokines, and prostaglandin E₂ levels

Cytokines and chemokines were evaluated with an enzyme-linked immunosorbent assay (ELISA), as described previously [16]. Briefly, cells were seeded and treated with sesamol for 1 h; then, the cells were stimulated with 1 μ g/ml LPS and incubated for 6 h to assay the levels of IL-6 and TNF- α , or they were cultured for 24 h to assay IL-1 β , MCP-1, and prostaglandin E₂ (PGE₂) production. The cytokines, chemokines, and PGE₂ in the medium were measured with specific ELISA kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The absorbance at 450 nm was measured with a multi-detection reader (Multiskan FC, Thermo).

Western immunoblot analysis

Cells (5×10^5 cells/ml) were treated with sesamol for 1 h in 6-well plates. Then, cells were stimulated with 1 μ g/ml LPS for 30 min to detect protein phosphorylation or for 24 h to detect the expression of total proteins. Cell lysate samples were separated on 8–10 % SDS polyacrylamide gels and transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The PVDF membranes were incubated overnight at 4 $^{\circ}$ C with primary antibodies against COX-2, HO-1, iNOS, Nrf2, I κ B- α , Lamin B1, phosphorylated-I κ B- α , p65 (Santa Cruz, CA, USA), AMPK, ERK1/2, p38, JNK, phosphorylated-AMPK, phosphorylated-ERK 1/2, phosphorylated-p38, phosphorylated-JNK,

(Millipore), and β -actin (Sigma). The PVDF membrane was washed with TBST buffer (150 mM NaCl, 10 mM Tris pH 8.0, 0.1 % Tween 20), and the membranes were incubated with HRP-conjugated secondary antibodies for 1 h at room temperature. Finally, the membranes were incubated with Luminol/Enhancer Solution (Millipore) to detect and quantify specific protein levels with the BioSpectrum 600 system (UVP, Upland, CA, USA).

RNA isolation and real-time PCR for gene expression

RNA was isolated with TRIzol reagent (Life Technologies, Carlsbad, CA, USA), and cDNA was synthesized with a cDNA synthesis kit (Life Technologies). cDNA gene expression was assayed with real-time PCR conducted on a spectrofluorometric thermal cycler (iCycler; Bio-Rad Laboratories, Hercules, CA, USA). Specific primers were designed as shown in Table 1.

Determination of reactive oxygen species (ROS) production

The cells were seeded in 96-well plates and treated with various concentrations of sesamol. Then, cells were stimulated with 1 μ g/ml LPS for 24 h. The supernatant was removed and cells were washed with PBS. Then, 20 μ M DCFH-DA was added to each well for 30 min at 37 $^{\circ}$ C. The supernatants were collected on black fluorometric plates, and the fluorescence was measured at 485 nm excitation

Table 1 Primers used in the experiments

Gene	Primers	(5–3' sequence)	GenBank accession number	Product size (bp)
IL-1 β	Forward	CACTACAGGCTCCGAGATGA	NM_008361	142
	Reverse	CGTTGCTTGGTTCTCCTTGT		
IL-6	Forward	AGGACCAAGACCATCCAATTCA	NM_031168	97
	Reverse	GCTTAGGCATAACGCACTAGG		
TNF- α	Forward	GCACCACCATCAAGGACTC	NM_013693	96
	Reverse	AGGCAACCTGACCACTCTC		
MCP-1	Forward	TTCCACAACCACCTCAAGCA	NM_011333	80
	Reverse	TTAAGGCATCACAGTCCGAGTC		
iNOS	Forward	TTCCACAACCACCTCAAGCA	NM_010927	83
	Reverse	TTAAGGCATCACAGTCCGAGTC		
COX-2	Forward	ACCAGCAGTCCAGTATCAGA	NM_011198	143
	Reverse	CAGGAGGATGGAGTTGTTGTAG		
β -Actin	Forward	AAGACCTCTATGCCAACACAGT	NM_007393	92
	Reverse	AGCCAGAGCAGTAATCTCCTTC		

and 528 nm emission on a Multi-Mode microplate reader (BioTek synergy HT, Bedfordshire, UKi).

Statistical analysis

All experimental analyses were compared with one-way analyses of variance (ANOVAs) and post hoc analyses with Dunnett's test. Data are expressed as the mean \pm standard deviation (SD) from at least three separate experiments. Differences were considered significant at $p < 0.05$.

Results

Effects of sesamol on the production of nitric oxide (NO) and PGE₂

Sesamol did not cause significant cell cytotoxicity at doses ≤ 100 μ M (Fig. 1b). All experiments used sesamol concentrations of 3–100 μ M. First, we assayed the sesamol effect on NO and PGE₂ production in LPS-stimulated RAW 264.7 cells. Sesamol significantly inhibited nitrite (a metabolite of NO) production in a concentration-dependent manner. Cells produced 46.8 ± 4.8 μ M nitrite with LPS alone; 44.3 ± 11.9 μ M with 3 μ M sesamol ($p = 0.12$); 36.4 ± 5.3 μ M with 10 μ M sesamol ($p < 0.05$); 27.5 ± 8.8 μ M with 30 μ M sesamol ($p < 0.01$); and 18.6 ± 5.3 μ M with 100 μ M sesamol ($p < 0.01$). The half maximal inhibitory concentration (IC₅₀) of sesamol was 38.4 ± 4.42 μ M (Fig. 1c). Sesamol also significantly suppressed PGE₂ production. Cells produced 10.1 ± 0.9 ng/ml PGE₂ with LPS; 9.5 ± 1.8 ng/ml with 3 μ M sesamol ($p = 0.21$);

8.3 ± 1.9 ng/ml with 10 μ M sesamol ($p = 0.08$); 6.7 ± 1.8 ng/ml with 30 μ M sesamol ($p < 0.01$); and 4.1 ± 1.3 ng/ml with 100 μ M sesamol ($p < 0.01$) (Fig. 1d).

Effects of sesamol on iNOS and COX-2 protein and mRNA expression

Next, we investigated whether the observed sesamol inhibition of NO and PGE₂ was related to iNOS and COX-2 expression. We found that sesamol significantly decreased iNOS and COX-2 protein expression in macrophages compared to LPS-stimulation (Fig. 2a, b). We used real-time PCR to assess gene expression, and we found that sesamol also significantly reduced iNOS and COX-2 mRNA expression in LPS-induced RAW 264.7 macrophages (Fig. 2c, d).

Sesamol inhibited production of proinflammatory cytokines

In RAW 264.7 cells, sesamol dose-dependently inhibited LPS-induced TNF- α production. Cells produced 12.8 ± 1.8 ng/ml TNF- α with LPS alone; 12.2 ± 1.3 ng/ml with 3 μ M sesamol ($p = 0.16$); 10.1 ± 2.6 ng/ml with 10 μ M sesamol ($p = 0.06$); 7.9 ± 1.5 ng/ml with 30 μ M sesamol ($p < 0.01$); and 6.8 ± 1.8 ng/ml with 100 μ M sesamol ($p < 0.01$) (Fig. 3a). Sesamol also significantly suppressed the levels of IL-1 β , IL-6, and MCP-1 (Fig. 3b–d). Real-time PCR analysis of gene expression showed that sesamol significantly decreased the expression of TNF- α , IL-1 β , IL-6, and MCP-1 compared to LPS alone in RAW 264.7 macrophages (Fig. 4).

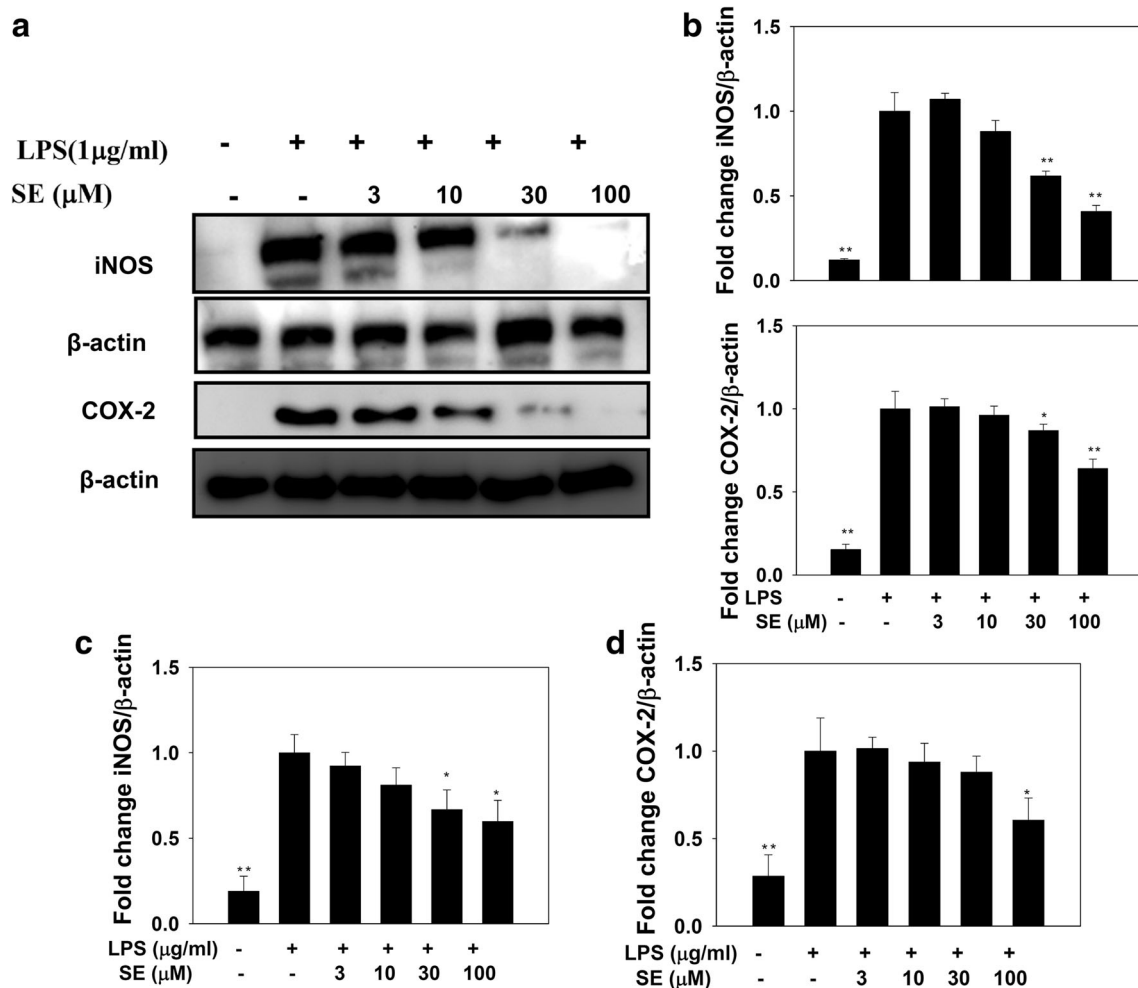


Fig. 2 Effects of sesamol on LPS-induced production of iNOS and COX-2. RAW 264.7 cells (10^6 cells/ml) were pretreated with the indicated concentrations of sesamol (SE) for 1 h and then stimulated with LPS (1 μ g/ml). **a**, **b** iNOS and COX-2 protein levels were detected on western blots. **b** Densitometric quantification of the fold

changes in protein levels, relative to β -actin protein (internal control). **c**, **d** Real-time PCR results show iNOS and COX-2 gene expression. The fold expression levels were calculated relative to the expression of β -actin (internal control). Data are presented as the mean \pm SD; * p < 0.05, ** p < 0.01, compared to LPS alone

Sesamol inhibited NF- κ B activation and I κ B α degradation in macrophages

The NF- κ B pathway is an important signaling pathway that regulates expression of inflammatory mediators [23]. Hence, we examined sesamol effects on the molecular mechanism of this pathway, where phosphorylation of I κ B- α releases NF- κ B (active subunit p65) for translocation into the nucleus. In unstimulated RAW 264.7 cells, NF- κ B (p65) was mostly distributed in the cytoplasm (Fig. 5a); exposure to LPS increased I κ B- α phosphorylation (Fig. 5a, b) and increased NF- κ B (p65) translocation into the nucleus (Fig. 5a, c, d). Our results showed that sesamol could significantly suppress I κ B- α phosphorylation, which

reduced the nuclear translocation of p65 compared to that induced by LPS alone in RAW 264.7 cells.

Effect of sesamol on phosphorylation of MAPK pathways

MAPK pathways can modulate the production of inflammatory mediators and cytokines in LPS-stimulated macrophages [24]. Therefore, we evaluated whether sesamol could suppress MAPK activation in LPS-stimulated macrophages. The results showed that sesamol inhibited the phosphorylation of ERK1/2, p38, and JNK, compared to that observed in LPS-stimulated macrophages (Fig. 6).

Fig. 3 The effects of sesamol on LPS-induced production of cytokines. ELISA results show the levels of **a** TNF- α , **b** IL-1 β , **c** IL-6, and **d** MCP-1. Cells (5×10^5 cells/ml) were pretreated with the indicated concentrations of sesamol (SE) for 1 h and then stimulated with LPS (1 μ g/ml). Cytokines secreted into the medium were collected for ELISA assays. The presented data are the mean \pm SD; * $p < 0.05$, ** $p < 0.01$, compared to LPS alone

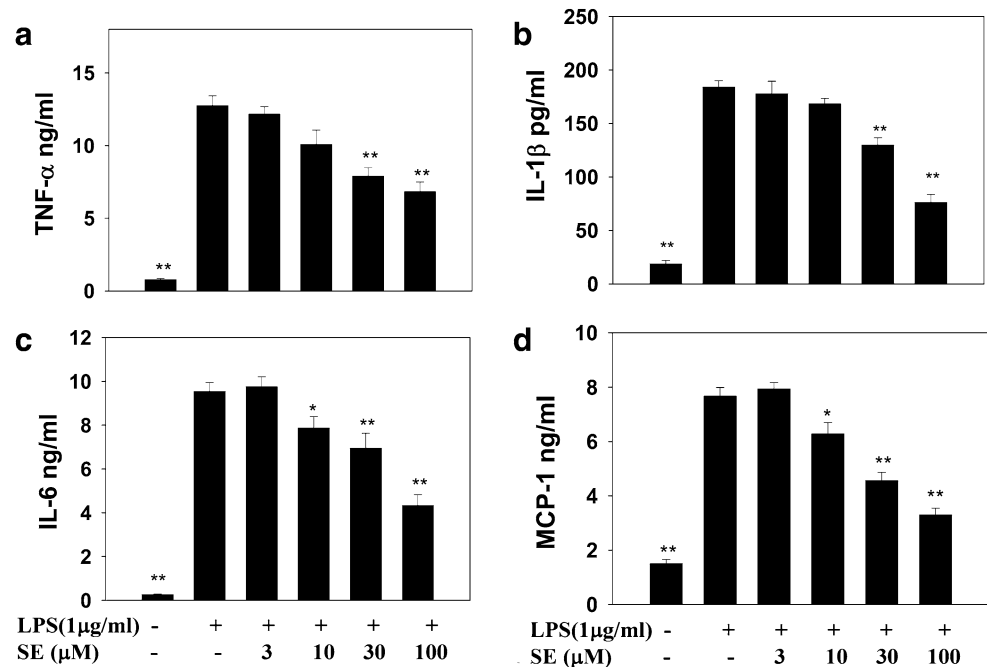
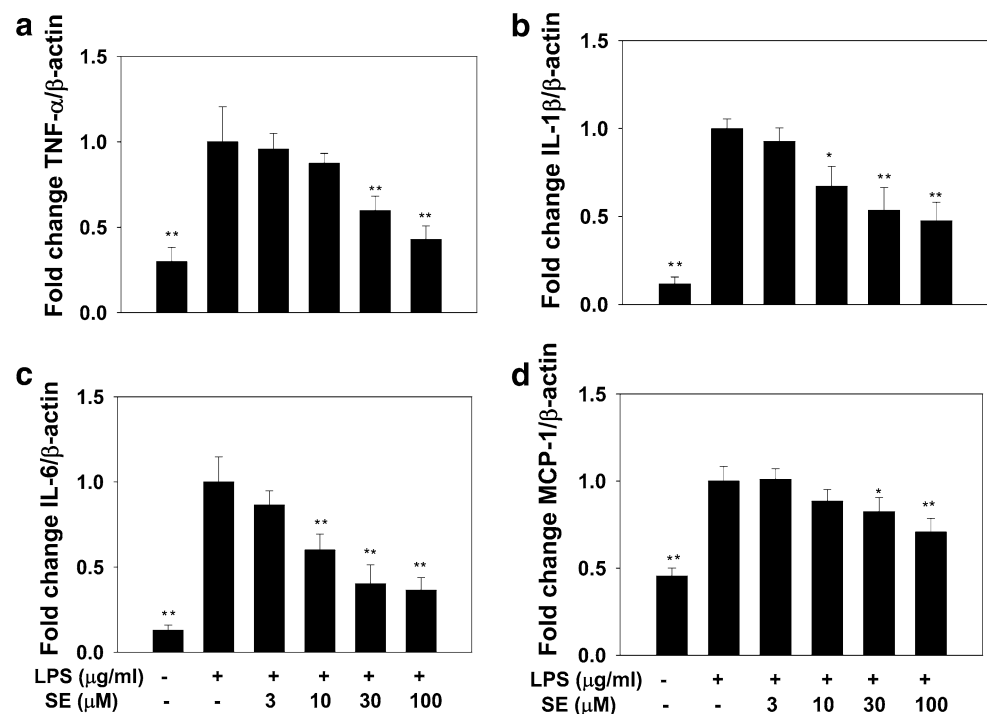


Fig. 4 Effects of sesamol on LPS-induced expression of proinflammatory cytokines and chemokines. RAW 264.7 cells (5×10^5 cells/ml) were pretreated with the indicated concentrations of sesamol (SE) for 1 h and then stimulated with LPS (1 μ g/ml) for 4 h. Real-time RT-PCR results show gene expression levels of **a** TNF- α , **b** IL-1 β , **c** IL-6, and **d** MCP-1. The fold expression levels were calculated relative to the level of β -actin (internal control). The data represent the mean \pm SD; * $p < 0.05$, ** $p < 0.01$, compared to LPS alone



Effect of sesamol on AMPK activation

We found that sesamol alone did not significantly increase AMPK phosphorylation in macrophages (Fig. 7a). However, LPS-stimulated macrophages could decrease AMPK phosphorylation. Interestingly, sesamol enhanced LPS-stimulated phosphorylation of AMPK in a concentration-dependent manner in macrophages (Fig. 7a). We also found that sesamol recovered AMPK activation and

suppressed TNF- α production when macrophages were treated with the AMPK inhibitor, compound C (Fig. 7b, c).

Sesamol enhanced HO-1 and Nrf2 expression

HO-1 is thought to have antioxidant effects [10]. Our results showed that sesamol significantly increased HO-1 production in macrophages (Fig. 8a). Sesamol also enhanced Nrf2 expression. In addition, we found that LPS

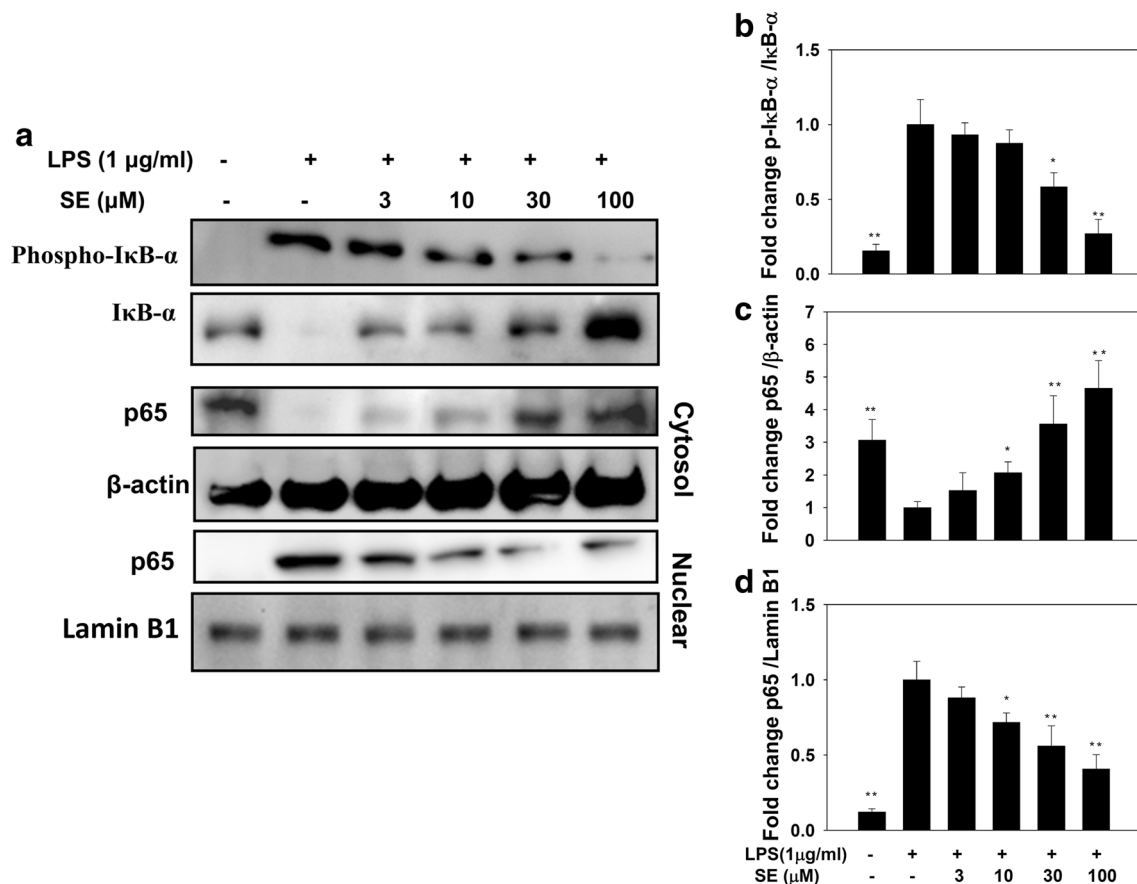


Fig. 5 Sesamol inhibited the LPS-induced nuclear translocation of NF- κ B in RAW 264.7 cells. Protein samples were analyzed on Western blots with specific antibodies. **a** Western blot shows the effects of the indicated concentrations of sesamol (SE) on the LPS-induced (*top row*) phosphorylation of I κ B- α , (*second row*) total I κ B- α levels, (*third row*) the cytosolic levels of p65, the catalytic subunit of NF- κ B, and (*bottom row*) the nuclear levels of NF- κ B (p65). Cells were incubated with sesamol for 1 h and then with LPS (1 μ g/ml) for

1 h. **b–d** The fold increases in protein levels were determined with densitometry and calculated relative to the internal controls. **b** Phosphorylated I κ B- α relative to total I κ B- α ; **c** NF- κ B in the cytosol, relative to β -actin in the cytosol; and **d** NF- κ B in the nucleus relative to Lamin B1 in the nucleus. Values represent the mean \pm SD of three independent experiments. * p < 0.05, ** p < 0.01, compared to LPS alone

stimulated increases in HO-1 and Nrf2 expression in macrophages. The addition of sesamol significantly enhanced LPS effects on HO-1 and Nrf2 production in macrophages (Fig. 8b).

Effect of sesamol on ROS production

ROS production was measured using microplate readers with fluorescence detection, and LPS could stimulate the production of ROS (Fig. 8c). However, sesamol significantly decreased ROS expression in LPS-stimulated RAW 264.7 cells.

Discussion

Sesamun indicum is an ancient crop with high oil content in the seeds [22]. Sesame oil is a common edible oil, high in vitamins, minerals, phyosterols, and polyunsaturated fatty

acids [17]. In China and India, people used sesame oil to ameliorate burns, and as a lubricant in massage [25]. In addition, sesame oil has an antimicrobial effect against *Staphylococcus*, and it suppresses fungal infections [20]. Other studies have reported that *Sesamun indicum* has potential antioxidant, anti-hypertensive, and anti-hyperlipidemic effects [18]. Recent phytochemical studies found that *Sesamun indicum* contained several lignans, including sesamol, sesamin, sesamolin, and sesaminol [20]. Sesamin was found to suppress chemokine production via the MAPK, PPAR- α , and NF- κ B pathways in LPS-activated human monocytes [26]. Other studies found that sesamol could decrease the levels of IL-1 β and TNF- α in LPS-induced lung injury in rats, and it attenuated IL-1 β and TNF- α production in LPS-treated macrophages [23, 27]. However, the anti-inflammatory mechanism of sesamol was not fully understood. In the present study, we found that sesamol suppressed the production of chemokines and

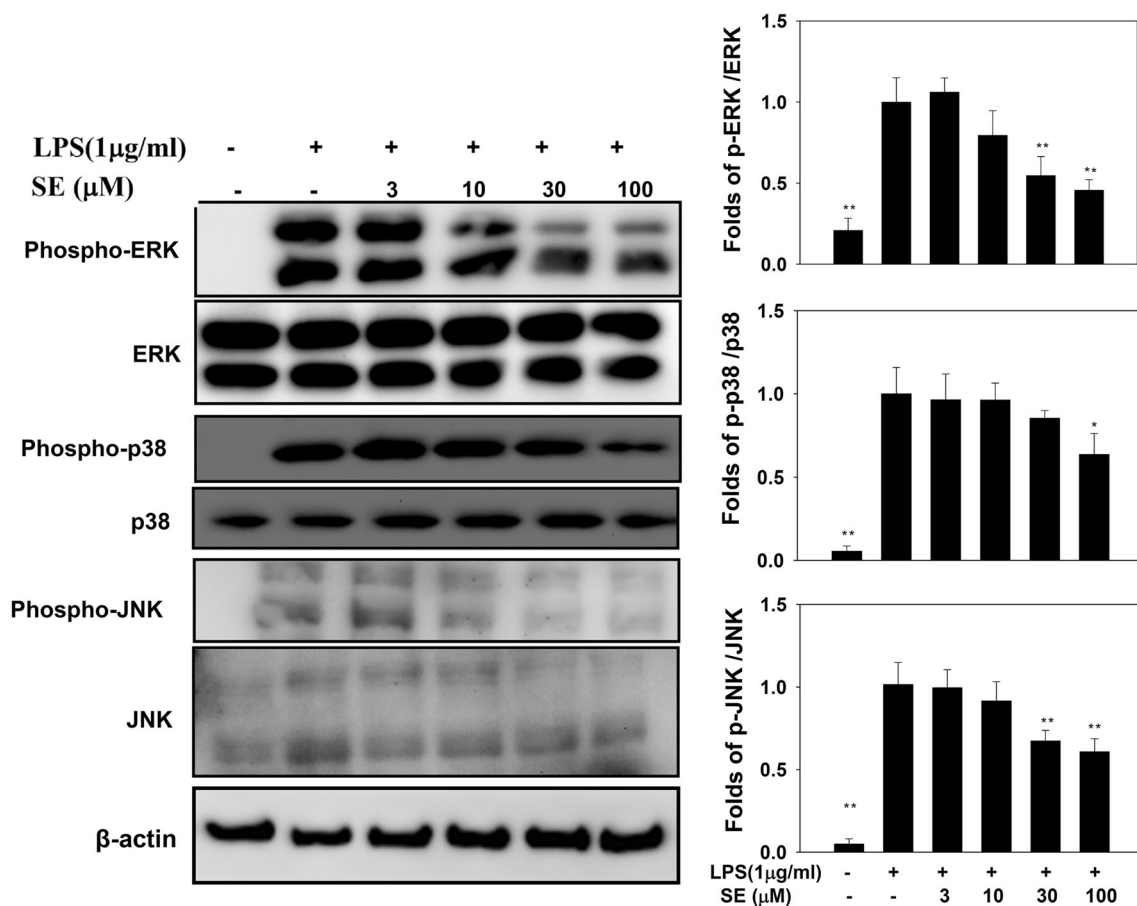


Fig. 6 Effect of sesamol on LPS-induced phosphorylation of MAPK pathway molecules. RAW 264.7 cells were pretreated with varying concentrations of sesamol (SE) for 1 h, then incubated with or without LPS (1 μg/ml) for 30 min. Protein samples were analyzed on Western blots with phospho-specific antibodies. Western blots show the effects of the indicated concentrations of sesamol (SE) on the

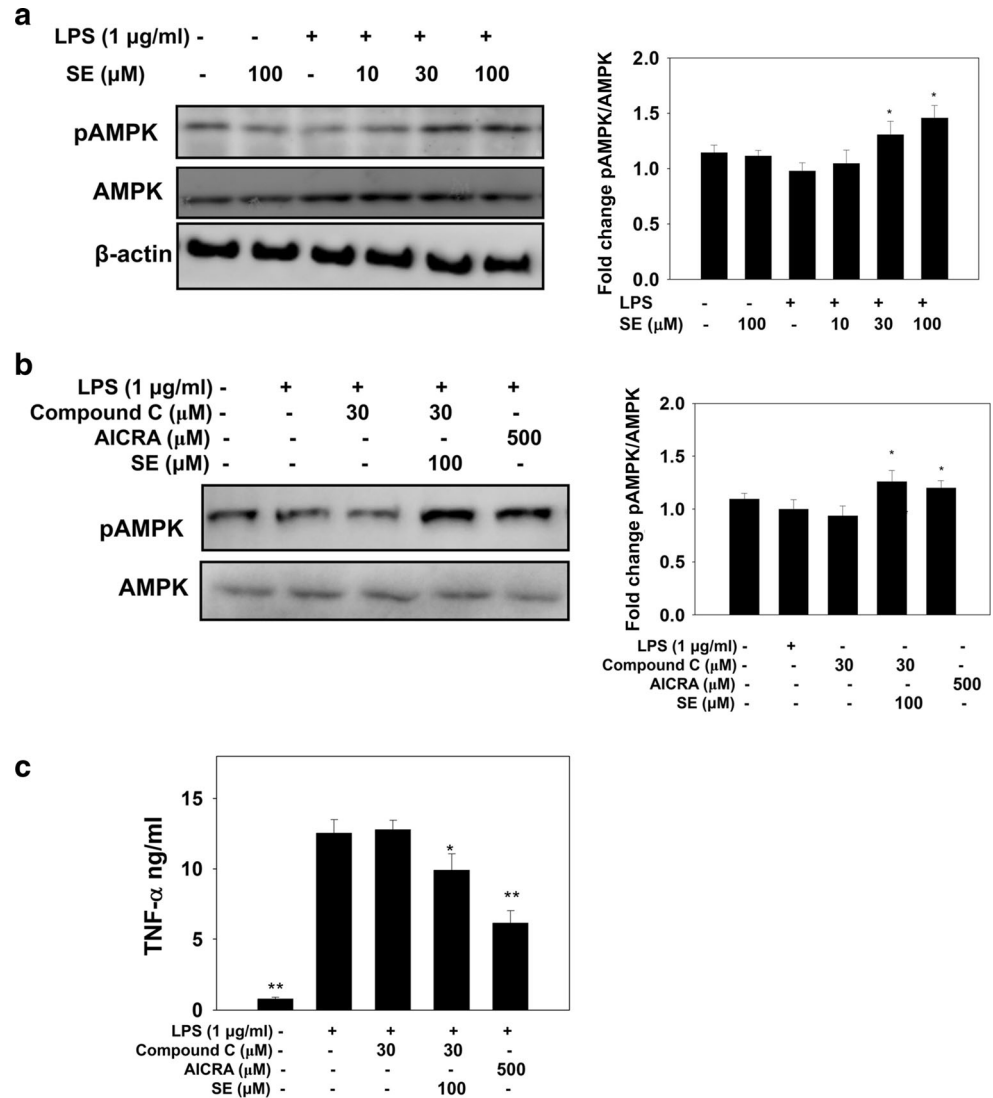
phosphorylation of (*top two rows*) ERK, (*middle two rows*) p38, and (*bottom two rows*) JNK. The fold changes in protein phosphorylation levels (p-) were determined with densitometry and calculated relative to the total levels of each protein. Densitometry values represent the mean ± SD of three independent experiments; * $p < 0.05$, ** $p < 0.01$, compared to LPS alone

proinflammatory cytokines, including IL-1 β , IL-6, TNF- α , and MCP-1, in LPS-stimulated murine macrophages. In addition, we showed that sesamol inhibited the release of inflammatory mediators, NO and PGE₂, and down-regulated iNOS and COX-2 mRNA and protein expression. We demonstrated that sesamol up-regulated AMPK activation and HO-1 protein expression in LPS-stimulated macrophages. We also found that sesamol significantly inhibited inflammatory processes and enhanced antioxidant-associated signaling pathways, including NF- κ B, p65, and Nrf2 nuclear translocation and MAPK phosphorylation. Therefore, we suggest that sesamol may ameliorate the inflammatory effect of LPS stimulation in macrophages.

LPS is a component of the cell wall of gram-negative bacteria. LPS induces inflammatory cells to release proinflammatory cytokines and mediators against bacterial infections [28]. LPS stimulated macrophages to express iNOS, which could catalyze L-arginine to produce nitric

oxide (NO) [5]. Some studies found that NO could stimulate macrophage activity to release high levels of proinflammatory cytokines, which could cause septic shock [29]. LPS also stimulated COX-2 expression in macrophages. COX-2 converts arachidonic acid to PGE₂, which increases the inflammatory response [30]. Recent studies found that natural compounds could suppress the productions of inflammatory mediators [16, 26]. Luo et al. found that astragalus polysaccharide could inhibit COX-2 and iNOS expression via NF- κ B signal pathway in LPS-induced microglial cells [31]. Lycopene also suppresses NO and IL-6 production by blocking the activation of MAPK and NF- κ B pathways in macrophages [32]. We previously found that sophoraflavanone G isolated from *Sophora flavescens* could suppress COX-2 and iNOS expression in LPS-stimulated macrophages [9]. In the present study, sesamol significantly reduced iNOS and NO production and also inhibited COX-2 and PGE₂ expression. These

Fig. 7 Effect of sesamol on LPS-induced phosphorylation of AMPK. RAW 264.7 cells were pretreated with varying concentrations of sesamol (SE) for 1 h and then incubated with or without LPS (1 μ g/ml) for 6 h. **a, b** Protein samples were analyzed on Western blots with phospho-specific antibodies. **b, c** AMPK protein levels were measured in the presence of an AMPK inhibitor (compound C) or an AMPK activator (AICRA) to compare effects on AMPK phosphorylation, and **c** TNF- α production. (**a, b right panels**) The fold changes in AMPK protein phosphorylation levels (p) were measured with densitometry and calculated relative to the total levels AMPK protein. **c** TNF- α was collected from the culture medium and measured with densitometry. All values represent the mean \pm SD of three independent experiments. * $p < 0.05$, ** $p < 0.01$, compared to LPS alone



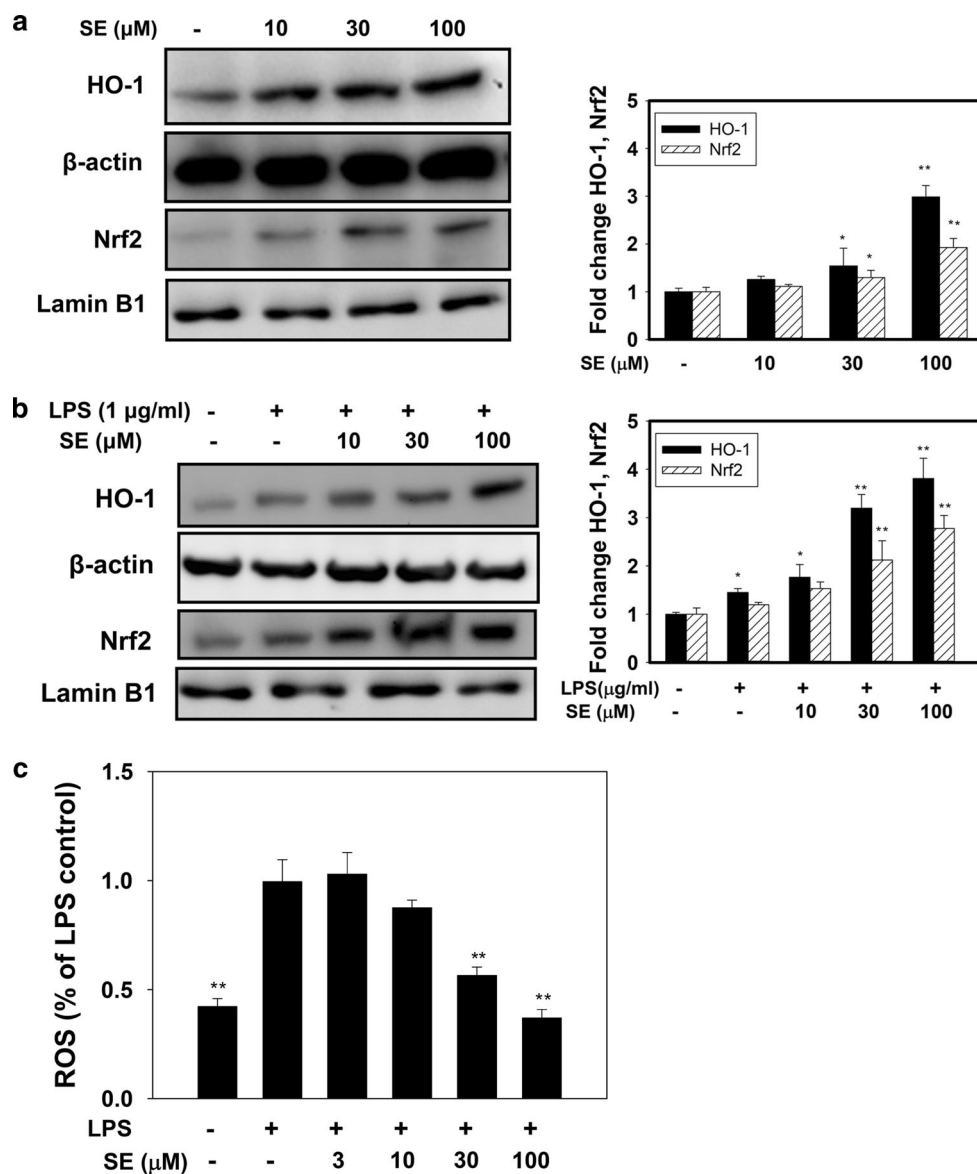
activities improved the inflammatory response in LPS-activated macrophages.

Activated macrophages can release multiple cytokines and chemokines that exacerbate the inflammatory response and lead to tissue injury [33]. IL-1 β is an important proinflammatory cytokine, which causes fever in response to a bacterial infection [34]. During the acute inflammatory response, IL-6 promotes C-reactive protein activation, which exacerbates inflammation and damages tissue [29]. In addition, when the liver releases large amounts of TNF- α , it causes hypotension and multiorgan dysfunction, which may even cause sepsis and death in response to bacterial infections [29]. LPS stimulates macrophages to release MCP-1, which attracts macrophages from the blood to areas of acute or chronic inflammation [35]. We previously found that casticin decreased the levels of proinflammatory cytokines via suppression of MAPK and NF- κ B signaling in LPS-stimulated macrophages [36]. In the present study,

sesamol suppressed IL-1 β , IL-6, and TNF- α production in LPS-induced macrophages. Hence, sesamol may be a natural anti-inflammatory agent that can attenuate the inflammatory response during bacterial infections.

HO-1 catalyzes the oxidation of heme and produces carbon monoxide in mammalian cells [10]. Several studies have suggested that HO-1 induced anti-inflammatory and antioxidative stress effects by inhibiting the upregulation of TLR-4 in LPS-activated macrophages [5, 11]. HO-1 also reduced iNOS and COX-2 production in LPS-activated macrophages [9]. Hence, promoting HO-1 expression could improve the inflammatory response in macrophages. Nrf2 is a transcription factor that translocates into the nucleus and stimulates HO-1 expression to promote an antioxidant response [37]. A previous study found that HO-1 expression could suppress the inflammatory response in TNF- α activated endothelial cells, and it also reduced lung inflammation in *Pseudomonas aeruginosa* infections [38].

Fig. 8 Effects of sesamol on LPS-induced HO-1 and Nrf2 protein expression. RAW264.7 cells (5×10^5 cells/ml) were treated with the indicated concentrations of sesamol (SE) for 24 h, in the (a) absence or (b) presence of LPS. **a, b** (Left panels) Western blots show HO-1 and Nrf2 protein levels in sesamol-treated cells. (Right panels) Fold changes in protein levels were calculated relative to β -actin (cytosol) and Lamin B1 (nucleus). **c** Sesamol inhibits ROS production in RAW 264.7 cell. The densitometry values represent the mean \pm SD of three independent experiments; * $p < 0.05$, ** $p < 0.01$, compared to the effect with no sesamol (a) or with LPS alone (b) and (c)



Liu et al. found that epigallocatechin gallate could promote antioxidant response against inflammation in human aortic endothelial cells [39]. Many studies have found that inflammation associated with oxidative stress to cause tissue damage [10, 40]. The study of Anselmi et al. found that oxidized-low-density lipoproteins could induce inflammatory response of vascular endothelial cells to induce more macrophage infiltration in the vessel wall [41]. Those activated macrophages would release inflammatory mediators to promote the development of coronary complex plaques in acute coronary syndromes. Inflammation stimulated the expression of oxidative stress that could cause tissue damages, including acute lung injury and thrombosis [42]. Hence, to enhance HO-1 expression would reduce the tissue damage by oxidative stress and ameliorate inflammatory response in chronic inflammatory diseases. Our

results showed that sesamol induced Nrf2 to translocate into the nucleus and promote HO-1 production in macrophages. We suggest that treating with sesamol could enhance HO-1 expression for antioxidant effects and inhibit production of inflammatory mediators.

AMPK was confirmed as a sensor of cellular energy that regulates lipid and glucose metabolism in adipocytes and hepatocytes [12]. AMPK could inhibit acetylCoA carboxylase activity to decrease lipid synthase [43]. Recent studies found that AMPK activation also decreased inflammatory responses in patients with metabolic syndrome [13]. AMPK was also found to have an anti-inflammatory effect in LPS-stimulated macrophages [14]. In the present study, we showed that sesamol could promote AMPK activation in LPS-stimulated macrophages. Compound C, an AMPK inhibitor, inhibited AMPK phosphorylation [43]. We found

that, in the presence of Compound C, sesamol could recover AMPK activation in macrophages. Sesamol may promote AMPK activity to protect the cell from damage during the LPS-induced inflammatory response. However, normal macrophages did not seem to require enhanced AMPK activation to regulate energy balance. In contrast, inflammatory cells require more energy to activate the inflammatory signaling pathway. Hence, we suggest that sesamol may ameliorate inflammatory responses via activation of AMPK in metabolic diseases.

MAPK regulates cell proliferation, and it increases the expression of iNOS and COX-2 in macrophages [44]. Tea tree oil components, including terpinen-4-ol and alpha-terpineol, could inhibit the production proinflammatory cytokines on human macrophages by suppressing ERK or p38 MAPK signaling pathways [45]. We found that sesamol could significantly inhibit phosphorylation of three members of the MAPK family, ERK 1/2, p38, and JNK.

In gram-negative bacterial infections, LPS binds to the macrophage surface molecule, CD14, which activates inflammatory signaling pathways [5]. The potent activity of LPS to produce inflammatory cytokines and mediators may be explained by its effect on the NF- κ B signaling pathway in macrophages. LPS induces I κ B phosphorylation, which releases the NF- κ B heterodimer to translocate into the nucleus and stimulate expression of inflammatory-associated genes [17]. In the present study, we confirmed that sesamol could suppress I κ B phosphorylation and decrease NF- κ B translocation into the nucleus. Hence, sesamol inhibited the expression of inflammatory mediators by suppressing the NF- κ B pathway.

In summary, this study demonstrated that sesamol significantly inhibited expression of proinflammatory cytokines and mediators by suppressing the activation of NF- κ B and MAPK pathways and promoting AMPK activation. Sesamol also displayed an antioxidant effect by activating the Nrf2/HO-1 pathway in LPS-activated murine macrophages. The anti-inflammatory effects of sesamol suggest that it may serve as a potential treatment for various inflammatory diseases in the future.

Acknowledgments This study was supported in part by grants from the Chang Gung Memorial Hospital (CMRPF1C0021-2 and CMRPF1B0131-3) and the Chang Gung University of Science and Technology (EZRP3D0071, EZRP3D0081 and EZRP3D0091).

Conflict of interest The authors declare that there are no conflicts of interest.

References

- White CR, Garber DW, Anantharamaiah GM. Anti-inflammatory and cholesterol-reducing properties of apolipoprotein mimetics: a review. *J Lipid Res.* 2014;55:2007–21.
- Bhatelia K, Singh K, Singh R. TLRs: linking inflammation and breast cancer. *Cell Signal.* 2014;26:2350–7.
- Wolff CH. Innate immunity and the pathogenicity of inhaled microbial particles. *Int J Biol Sci.* 2011;7:261–8.
- Krishnan J, Selvarajoo K, Tsuchiya M, Lee G, Choi S. Toll-like receptor signal transduction. *Exp Mol Med.* 2007;39:421–38.
- Park BS, Lee JO. Recognition of lipopolysaccharide pattern by TLR4 complexes. *Exp Mol Med.* 2013;45:e66.
- Oeckinghaus A, Hayden MS, Ghosh S. Crosstalk in NF- κ B signaling pathways. *Nat Immunol.* 2011;12:695–708.
- Hinz M, Scheidereit C. The I κ B kinase complex in NF- κ B regulation and beyond. *EMBO Rep.* 2014;15:46–61.
- Bony E, Boudard F, Dussossoy E, Portet K, Brat P, Giaimis J, et al. Chemical composition and anti-inflammatory properties of the unsaponifiable fraction from awara (*Astrocyarium vulgare* M.) pulp oil in activated J774 macrophages and in a mice model of endotoxic shock. *Plant Foods Hum Nutr.* 2012;67:384–92.
- Wun ZY, Lin CF, Huang WC, Huang YL, Xu PY, Chang WT, et al. Anti-inflammatory effect of sophoraflavanone G isolated from *Sophora flavescens* in lipopolysaccharide-stimulated mouse macrophages. *Food Chem Toxicol.* 2013;62:255–61.
- Araujo JA, Zhang M, Yin F. Heme oxygenase-1, oxidation, inflammation, and atherosclerosis. *Front Pharmacol.* 2012;3:119.
- Zhu X, Fan WG, Li DP, Kung H, Lin MC. Heme oxygenase-1 system and gastrointestinal inflammation: a short review. *World J Gastroenterol.* 2011;17:4283–8.
- Hardie DG. AMPK: positive and negative regulation, and its role in whole-body energy homeostasis. *Curr Opin Cell Biol.* 2014;33:1–7.
- Kim J, Kwak HJ, Cha JY, Jeong YS, Rhee SD, Kim KR, et al. Metformin suppresses lipopolysaccharide (LPS)-induced inflammatory response in murine macrophages via activating transcription factor-3 (ATF-3) induction. *J Biol Chem.* 2014;289:23246–55.
- Ji G, Zhang Y, Yang Q, Cheng S, Hao J, Zhao X, et al. Genistein suppresses LPS-induced inflammatory response through inhibiting NF- κ B following AMP kinase activation in RAW 264.7 macrophages. *PLoS One.* 2012;7:e53101.
- Sarić A, Sobocanec S, Balog T, Kusić B, Sverko V, Dragović-Uzelac V, et al. Improved antioxidant and anti-inflammatory potential in mice consuming sour cherry juice (*Prunus Cerasus* cv. Maraska). *Plant Foods Hum Nutr.* 2009;64:231–7.
- Chang WT, Huang WC, Liou CJ. Evaluation of the anti-inflammatory effects of phloretin and phlorizin in lipopolysaccharide-stimulated mouse macrophages. *Food Chem.* 2012;134:972–9.
- Visavadiya NP, Soni B, Dalwadi N. Free radical scavenging and antiatherogenic activities of *Sesamum indicum* seed extracts in chemical and biological model systems. *Food Chem Toxicol.* 2009;47:2507–15.
- Dhibi M, Mechri B, Cheraif I, Hammami M. Trans-Fatty acid isomers in two sesame (*Sesamum indicum* L.) seed byproducts under processing. *J Agric Food Chem.* 2010;58:12210–5.
- Li C, Miao H, Wei L, Zhang T, Han X, Zhang H. Association mapping of seed oil and protein content in *Sesamum indicum* L. using SSR markers. *PLoS One.* 2014;9:e105757.
- Dar AA, Arumugam N. Lignans of sesame: purification methods, biological activities and biosynthesis—a review. *Bioorg Chem.* 2013;50:1–10.
- Liu Z, Xiang Q, Du L, Song G, Wang Y, Liu X. The interaction of sesamol with DNA and cytotoxicity, apoptosis, and localization in HepG2 cells. *Food Chem Toxicol.* 2013;141:289–96.
- Kumar N, Mudgal J, Parihar VK, Nayak PG, Kuttly NG, Rao CM. Sesamol treatment reduces plasma cholesterol and triacylglycerol levels in mouse models of acute and chronic hyperlipidemia. *Lipids.* 2013;48:633–8.

23. Chu PY, Chien SP, Hsu DZ, Liu MY. Protective effect of sesamol on the pulmonary inflammatory response and lung injury in endotoxemic rats. *Food Chem Toxicol.* 2010;48:1821–6.
24. Ayroldi E, Cannarile L, Migliorati G, Nocentini G, Delfino DV, Riccardi C. Mechanisms of the anti-inflammatory effects of glucocorticoids: genomic and nongenomic interference with MAPK signaling pathways. *FASEB J.* 2012;26:4805–20.
25. Shenoy RR, Sudheendra AT, Nayak PG, Paul P, Kutty NG, Rao CM. Normal and delayed wound healing is improved by sesamol, an active constituent of *Sesamum indicum* (L.) in albino rats. *J Ethnopharmacol.* 2011;133:608–12.
26. Hsieh CC, Kuo CH, Kuo HF, Chen YS, Wang S, Chao D, et al. Sesamin suppresses macrophage-derived chemokine expression in human monocytes via epigenetic regulation. *Food Funct.* 2014;5:2494–500.
27. Chu PY, Hsu DZ, Hsu PY, Liu MY. Sesamol down-regulates the lipopolysaccharide-induced inflammatory response by inhibiting nuclear factor-kappa B activation. *Innate Immun.* 2010;16:333–9.
28. Guha M, Mackman N. LPS induction of gene expression in human monocytes. *Cell Signal.* 2001;13:85–94.
29. Chong DL, Sriskandan S. Pro-inflammatory mechanisms in sepsis. *Contrib Microbiol.* 2011;17:86–107.
30. Wu KK. Control of COX-2 and iNOS gene expressions by aspirin and salicylate. *Thromb Res.* 2003;110:273–6.
31. Luo T, Qin J, Liu M, Luo J, Ding F, Wang M, et al. Astragalus polysaccharide attenuates lipopolysaccharide-induced inflammatory responses in microglial cells: regulation of protein kinase B and nuclear factor- κ B signaling. *Inflamm Res.* 2015;64:205–12.
32. Feng D, Ling WH, Duan RD. Lycopene suppresses LPS-induced NO and IL-6 production by inhibiting the activation of ERK, p38MAPK, and NF- κ B in macrophages. *Inflamm Res.* 2010;59:115–21.
33. Devkota S, Chang EB. Nutrition, microbiomes, and intestinal inflammation. *Curr Opin Gastroenterol.* 2013;29:603–7.
34. Dinarello CA. The IL-1 family and inflammatory diseases. *Clin Exp Rheumatol.* 2002;20:S1–13.
35. Karta MR, Gavala ML, Curran CS, Wickert LE, Keely PJ, Gern JE, et al. LPS modulates rhinovirus-induced chemokine secretion in monocytes and macrophages. *Am J Respir Cell Mol Biol.* 2014;51:125–34.
36. Liou CJ, Len WB, Wu SJ, Lin CF, Wu XL, Huang WC. Casticin inhibits COX-2 and iNOS expression via suppression of NF- κ B and MAPK signaling in lipopolysaccharide-stimulated mouse macrophages. *J Ethnopharmacol.* 2014;158:310–6.
37. Keum YS, Choi BY. Molecular and chemical regulation of the Keap1-Nrf2 signaling pathway. *Molecules.* 2014;19:10074–89.
38. Zhou H, Lu F, Latham C, Zander DS, Visner GA. Heme oxygenase-1 expression in human lungs with cystic fibrosis and cytoprotective effects against *Pseudomonas aeruginosa* in vitro. *Am J Respir Crit Care Med.* 2004;170:633–40.
39. Liu PL, Liu JT, Kuo HF, Chong IW, Hsieh CC. Epigallocatechin gallate attenuates proliferation and oxidative stress in human vascular smooth muscle cells induced by interleukin-1 β via heme oxygenase-1. *Mediators Inflamm.* 2014;2014:523684.
40. Paine A, Eiz-Vesper B, Blasczyk R, Immenschuh S. Signaling to heme oxygenase-1 and its anti-inflammatory therapeutic potential. *Biochem Pharmacol.* 2010;80:1895–903.
41. Anselmi M, Garbin U, Agostoni P, Fusaro M, Pasini AF, Nava C, et al. Plasma levels of oxidized-low-density lipoproteins are higher in patients with unstable angina and correlated with angiographic coronary complex plaques. *Atherosclerosis.* 2006;185:114–20.
42. Pashkow FJ. Oxidative stress and inflammation in heart disease: do antioxidants have a role in treatment and/or prevention? *Int J Inflamm.* 2011;2011:514623.
43. Takeda J, Park HY, Kunitake Y, Yoshiura K, Matsui T. Theaflavins, dimeric catechins, inhibit peptide transport across Caco-2 cell monolayers via down-regulation of AMP-activated protein kinase-mediated peptide transporter PEPT1. *Food Chem Toxicol.* 2013;138:2140–5.
44. Huang P, Han J, Hui L. MAPK signaling in inflammation-associated cancer development. *Protein Cell.* 2010;1:218–26.
45. Nogueira MN, Aquino SG, Rossa Junior C, Spolidorio DM. Terpinen-4-ol and alpha-terpineol (tea tree oil components) inhibit the production of IL-1 β , IL-6 and IL-10 on human macrophages. *Inflamm Res.* 2014;63:769–78.