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Research Paper

Anti-inflammatory effect of *Rhus verniviflua* Stokes by suppression of iNOS-mediated Akt and ERK pathways: in-vitro and in-vivo studies

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Abstract

Objectives *Rhus verniciflua* Stokes (RVS), which has valuable medicinal properties, has for many years been prescribed for inflammation in east Asian medicine. Recent studies suggest that RVS has potent antioxidative, antitumor and anti-inflammatory properties.

Methods In this study, the anti-inflammatory effects of RVS *in vitro* and *in vivo* were investigated. The ethanol extract from RVS was partitioned with different solvents in order of increasing polarity.

Key findings Among the various extracts, the n-butanol extract displayed the most potent activity against nitric oxide and reactive oxygen species. The n-butanol extract also significantly regulates expression of nitric oxide synthase, which inhibits nitric oxide production at the transcriptional level in activated macrophages. Immunoblot analysis also showed that n-butanol extract suppresses the phosphorylation of extracellular signal-regulated kinase and Akt, suggesting that nitric oxide synthase suppression might be mediated via the extracellular signal-regulated kinase and Akt signaling pathways. This study also investigated whether n-butanol exerts an anti-inflammatory effect in an animal model. n-butanol extract significantly reduces carrageenan-induced mouse paw edema at 5 h.

Conclusions These results suggest that RVS could be a promising candidate agent for inflammation prevention and combination therapy with anti-inflammatory drugs.

Keywords extracellular signal-regulated kinase; nitric oxide synthase; *Rhus verniciflua* Stokes; v-akt murine thymoma viral oncogene homolog 1

Introduction

Non-steroidal anti-inflammatories, steroids and antihistamines were developed as a result of the discovery that the treatment of inflammatory diseases is largely based on interrupting the synthesis or action of critical mediators that drive the host's response to injury. It has also been proven that medicinal herbs exhibit anti-inflammatory effects via inhibition of the inflammatory signal pathway. *Rhus verniciflua* Stokes (RVS) has traditionally been used as an ingredient in Korean and Chinese medicines used to treat gastritis, stomach cancer and arteriosclerosis.[1] Recent studies have shown that *Rhus verniciflua* exerts antioxidative, antitumor and anti-inflammatory effects, and its pharmaceutical activities mainly come from their abundant phenolic content, including compounds such as butein, fustin and quercetin.[2–5] In addition, the effects of RVS on pro-inflammatory cytokines has recently been elucidated.[4] Pro-inflammatory cytokines such as TNF-α and interleukins have been known to play a critical role in inflammation, their effects involved with the migration and activation of leukocytes.

In Oriental medicine, anti-inflammatory drugs are usually used as prescriptions based on combinations of Oriental medicine or semi-purified extracts rather than as a single drug. In addition, extracts from plants and even the polymeric nanomicelles from the extracts have been examined for effects on various diseases, including their anti-inflammatory effects.[6,7] In this study, we prepared semi-purified extracts from RVS in order of increasing solvent

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polarity. We examined whether the extract had anti-inflammatory effects *in vitro* and *in vivo*. Our study has revealed that semi-purified extract from RVS inhibits lipopolysaccharide (LPS)-induced inflammation through the blocking of the extracellular signal-regulated kinase (ERK) and v-akt murine thymoma viral oncogene homolog 1 (Akt) pathways in macrophages, and demonstrated that the extract inhibits carrageenan-induced mouse-paw edema. To our knowledge, our study is the first to investigate the effects of a standardized RVS on the updated signaling pathways in macrophage RAW 264.7 cells and in an in-vivo anti-inflammatory study. We suggest that RVS may contribute to reducing inflammation.

Materials and Methods

Reagents

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, penicillin and streptomycin were purchased from Gibco Life Technologies (MD, USA). Cyclo-oxygenase (COX)-2, nitric oxide synthase (iNOS) and the peroxidase-conjugated secondary antibody were purchased from Santa Cruz Biotechnology (CA, USA). Enzyme immunoassay kits used for the determination of nitric oxide (NO) were obtained from Assay Designs, Inc. (MI, USA) and all other chemicals were purchased from Sigma-Aldrich Co. (MO, USA). RVS was purchased from Omniherb (Yeongcheon, South Korea) and a voucher specimen was deposited in the herbarium of the College of Oriental Medicine of Kyunghee University, Seoul, Korea.

Extraction and fractionation

Dried and finely powdered RVS bark was extracted twice by repeated sonication (30 min) with 80% ethanol. The 80% ethanol extract was filtered and dried using a vacuum evaporator. Ethanol extract dissolved in distilled water was extracted with diethyl ether (Et_2O), chloroform ($CHCl_3$), ethyl acetate (EtOAc) and n-butanol (BuOH) in order of increasing polarity. The yields of the extract were (w/w) diethyl ether fraction 2.46%, $CHCl_3$ fraction 0.35%, EtOAc fraction 0.81%, BuOH fraction 1.62%, water fraction 0.53%. Total phenolic content in the RVS extract was determined using Folin–Ciocalteu's reagent[8] and total flavonoid content was determined by the colorimetric method.[9]

Di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) radical-scavenging activity

The antioxidant activities of extracts and positive control were assessed on the basis of the radical-scavenging effect of the stable di(phenyl)-(2,4,6-trinitrophenyl)iminoazanium (DPPH) free radical. Extracts (20 µl) were added to 180 µl of DPPH (100 µm) solution in a 96-well microplate. After incubation at 37°C for 20 min, the absorbance of each solution was determined at 540 nm using an ELISA microplate reader (Model 550; Bio-Rad Laboratories Inc., CA, USA). Radical-scavenging activity (%) = $[1-(A_{sample}/A_{control})] \times 100$

Cell culture

Murine macrophage RAW 264.7 cells were obtained from the Korea Cell Line Bank (Seoul, Korea), and cultured in DMEM medium supplemented with 2 mM glutamine, antibiotics (100 U/ml of penicillin A and 100 µg/ml of streptomycin) and 10% heat-inactivated fetal bovine serum, and were maintained at 37°C in a 5% CO_2 humidified incubator.

(3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) cell viability assay

The cell viability assay was performed using (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) reagent. The MTS test is based on the capacity of viable cells to metabolize (via the mitochondrial succinate dehydrogenase) a yellow tetrazolium salt (MTS) in the presence of phenazine methosulfonate, acting as an electron coupling agent, to a purple formazan, which is directly soluble in tissue culture medium. The formazan can be measured by the amount of 490 nm absorbance, which is proportional to the number of living cells. The cells were cultured in a 96-well plate at a concentration of 5×10^4 cells/well. After 24 h of preconditioning, culture medium was aspirated and the cells were exposed to a variety of concentrations of sample for 24 h. Subsequently, 20 µl of MTS dye (1 mg/ml) was added to the cultures and incubated for 2 h at 37°C. The index of cell viability was calculated by measuring the optical density[10] of color produced by the MTS dye reduction at 490 nm.

Nitrite assay

Cells (5 × 104 cells/well) were pretreated with each extract (20 µg/ml) for 30 min and stimulated with LPS (1 µg/ml) for 18 h. The nitrite concentration in the medium is an indicator of NO production from the Griess reaction. One hundred microlitres of each supernatant from each well was transferred to another 96-well plate and 100 µl of the Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride in water) were added. The mixtures were incubated at room temperature for 10 min. The absorbance of the mixtures (at 550 nm) was determined with an ELISA plate reader.

Determination of production of reactive oxygen species

The molecule 2′,7′-dichlorofluorescein diacetate (DCHF-DA) is freely permeable in cells and, after incorporation into cells, is converted into the fluorescent 2,7-dichlorofluorescein (DCF) by oxidative substances. Therefore, DCHF-DA reveals the intracellular production of redox-active substances and it has been widely used to investigate oxidative damage in intact cells. Briefly, cells (5 × 104 cells/well) were pretreated with each extract (20 µg/ml) for 30 min and stimulated with LPS (1 µg/ml) for 18 h. After reaction, cells were incubated with 50 mM of fluorescent probe DCHF-DA for 30 min at 37°C. At the end of the oxidation treatment, cells were incubated with 50 µM of fluorescent probe DCHF-DA for 30 min at 37°C. The degree of fluorescence, corresponding to intracellular reactive oxygen species (ROS), was determined using Fluoroscan Ascent FL (Type 374, Labsytems, Finland).

Western blotting analysis

Cells (5×10^4) were obtained by centrifugation at 13 000*g* for 15 min at 4°C. Proteins were extracted by cell lysis buffer (50 mM HEPES pH 7.0, 250 mM sodium chloride, 5 mM EDTA, 0.1% Nonidet P-40, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol, 5 mM sodium fluoride and 0.5 mM sodium orthovanadate). Equal amounts of protein (40 μg) from the cell lysates were boiled for 5 min in SDS-PAGE sample buffer, resolved by 10% SDS-PAGE, transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA, USA) at 80 V for 1.5 h and visualized by Western blotting and chemiluminescence.

Reverse transcriptase polymerase chain reactions

Cells were collected by centrifugation and RNA was extracted using the Invitrogen kit according to the manufacturer's instructions. Primers were designed for reverse transcriptase-polymerase chain reactions (RT-PCR) for iNOS and COX-2. The sense and antisense primers for iNOS were 5′-AATGGCAACATCAGGTCGGCCATCACT-3′ and 5′-GCTGTGTGTCACAGAAGTCTCGAACTC-3′, respectively, and the sense and antisense primers for COX-2 were 5′-GGAGAGACTATCAAGATAGT-3′ and 5′-ATGGTCAGTAGACTTTTACA-3′, respectively. The 1 μg of cDNA was amplified by PCR for 20 cycles of denaturation (95°C for 1 min), annealing (iNOS, 40°C for 1 min or COX-2, 60°C for 1 min), polymerization (72°C for 1 min), and a final elongation step of 5 min at 72°C. The PCR products were separated by 2.5% agarose gel electrophoresis and visualized by ethidium bromide staining and UV irradiation.

Animals

Imprinting control region mice were housed in plastic cages under controlled conditions (temperature: 20 ± 2°C, humidity: 40–60%, 12 h light/dark cycle) and acclimatized for 1 week. For 24 h before the experiment only water was offered to the animals. All animal experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* established by the Korea National Institute of Health. Ethical approval was obtained from the Institutional Review Board of Kyunghee University.

Carrageenan-induced mouse paw edema

Carrageenan-induced mice paw edema was examined according to the previous method.[11] Before treatment, the average volume (V_0, basal volume) of the right paw of each animal was determined using a dial thickness gauge. The different groups were treated orally with BuOH extract (100 mg per kilogram of body weight) and saline control. All samples were dissolved in DMSO. One hour after administration, paw edema was induced by injection of 50 μl of 1% carrageenan into the right hind paw. The paw volumes were measured at 5 h after the injection, and the volume (V_t) of the edema was measured with a plethysmometer.

Liquid chromatography–mass spectrometry analysis

An Agilent (Palo Alto, CA) 1100 series quadrupole liquid chromatography–mass spectroscopy (LC–MS) with an atmospheric pressure chemical ionization interface was used in negative and positive ionization mode. Data were collected using Chemstation software version A.09.03. A GEMINI 5-μm C_{18} 110Å column (150 mm × 4.60 mm) (Phenomenex, CA) was used with an injection volume of 10 μl for the HPLC separation. The mobile phases consisted of (A) water and (B) methanol at a flow rate of 0.7 ml/min. The gradient of the mobile phases (A : B) for separation was 0–70 min (95 : 5 to 10 : 90). Fustin, butein, fisetin and sulfuretin were used as standards. Mass spectrometry was operated with an electrospray ionization source and positive mode.

Statistical analyses

All experimental data were examined by analyses of the variance (ANOVA) from triplicate determinations, and significant differences among the means were assumed at $P < 0.05$. Further statistical analyses were performed using a non-parametric test and the Statistical Analysis System (SAS Institute Inc., Cary, NC, USA).

Results

Extraction and fraction of RVS

RVS was extracted with 80% ethanol and partitioned successively with Et_2O, $CHCl_3$, EtOAc, BuOH and aqueous fractions. Table 1 shows the total phenolics and flavonoid contents and the DPPH radical-scavenging activities of the semi-purified fractions from RVS. Among the extracts, the EtOAc extract contained the highest quantity of phenolics (34.22 g/100 g) and flavonoids (1.89 g/100 g), followed by the BuOH and water extracts. The DPPH radical-scavenging activities of the solvent fractions decreased in the order EtOAc fraction (EC50 = 8.39 μg/ml) > BuOH (16.07 μg/ml) fraction > Et_2O (18.10 μg/ml) > $CHCl_3$ fraction (44.66 μg/ml). The radical scavenging actions are related to their total phenolic contents.

Effect of RVS on cell viability and NO and ROS production

First, the effect of RVS extracts on cell viability was evaluated. No significant cytotoxicity was observed when the cells were exposed to a maximum of 20 μg/ml for 24 h (Figure 1a). However, higher concentrations (>20 μg/ml) of ether and

Table 1 Total phenolics, flavonoids and DPPH radical-scavenging activity of various solvent extracts from *Rhus vernicifera* stokes

Extracts	Total phenolics	Total flavonoids	DPPH (EC_{50}, μg/ml)
Diethyl ether	21.56 ± 0.10	N/A	18.10 ± 0.05
Chloroform	13.18 ± 0.26	N/A	44.66 ± 0.12
Ethyl acetate	34.22 ± 0.05	1.89 ± 0.01	8.39 ± 0.09
n-butanol	20.81 ± 1.64	1.63 ± 0.04	16.07 ± 0.07
Water	5.10 ± 0.10	1.19 ± 0.01	>100

Each value is the mean ± SEM. EC_{50} values were obtained and signify the concentration of sample necessary to scavenge 50% of DPPH radicals. Total phenolics; g/100 g of extract, dry weight. EC, effective concentration; N/A, not available.

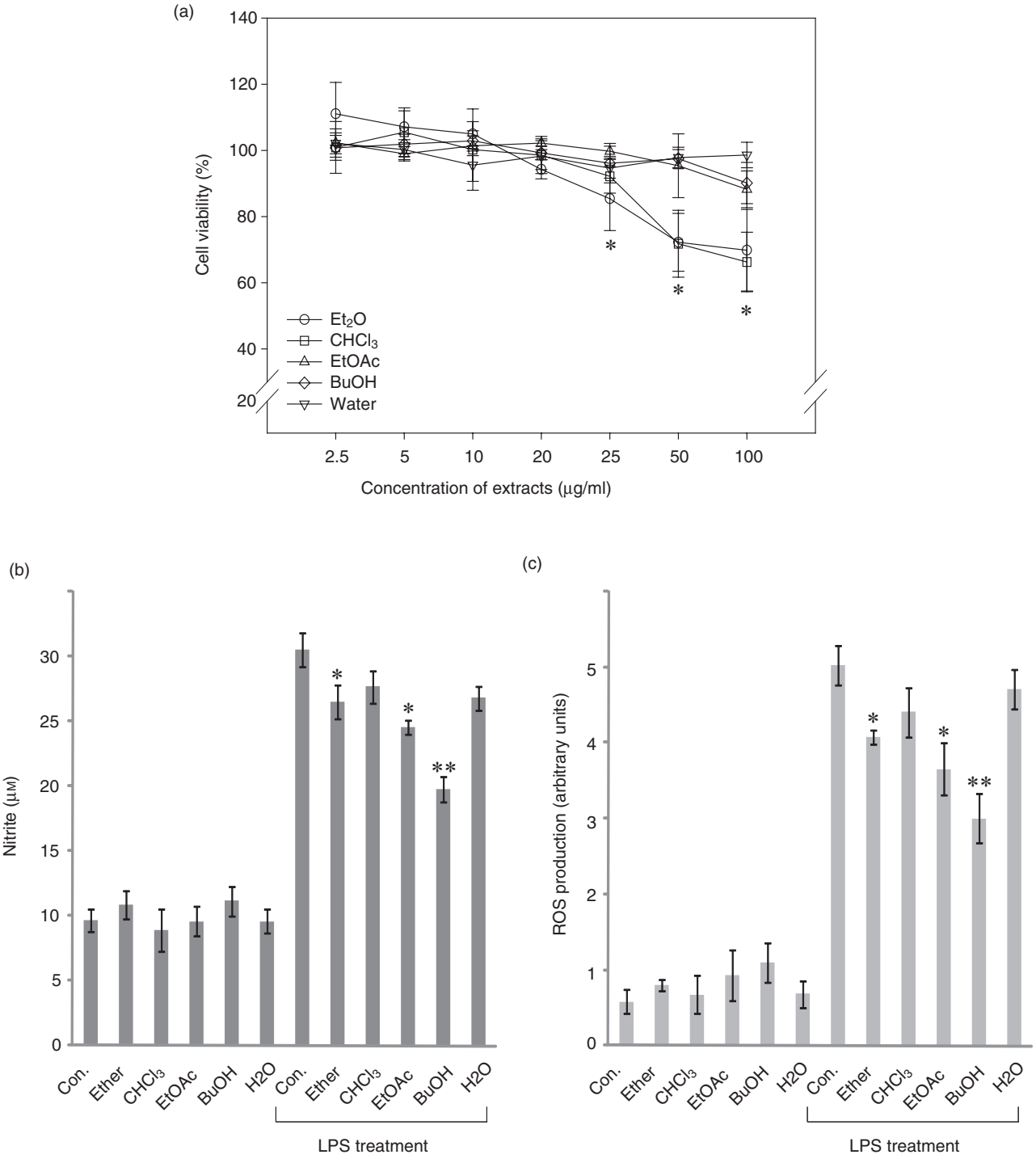

Figure 1 Effects of various solvent extracts on LPS-induced nitrite and ROS production in RAW264.7 macrophages. (a) Cell viability was tested in activated macrophage. Cell viabilities were determined using MTS assays. Significantly reduced cell viability is shown at the higher concentration than 20 μg/ml of ether and chloroform fractions (*$P < 0.05$, $n = 3$). Nitrite (b) and ROS (c) production were measured by the Griess reaction and the DCHF-DA dye (a free radical recognizing dye). Significant difference (*$P < 0.005$, **$P < 0.0001$, $n = 3$) is compared to LPS-treated control.

$CHCl_3$ fractions reduced cell viability. Due to the cytotoxicity at high concentrations of the Et_2O and $ChCl_3$ fractions, 20 μg/ml of each extract was used in the NO and ROS production. Since the inhibition of inflammatory mediators such as NO and ROS could be employed as a criterion for evaluation of anti-inflammatory effects, this study determined whether RVS extracts inhibit NO and ROS production in LPS-induced macrophages. The results showed that the

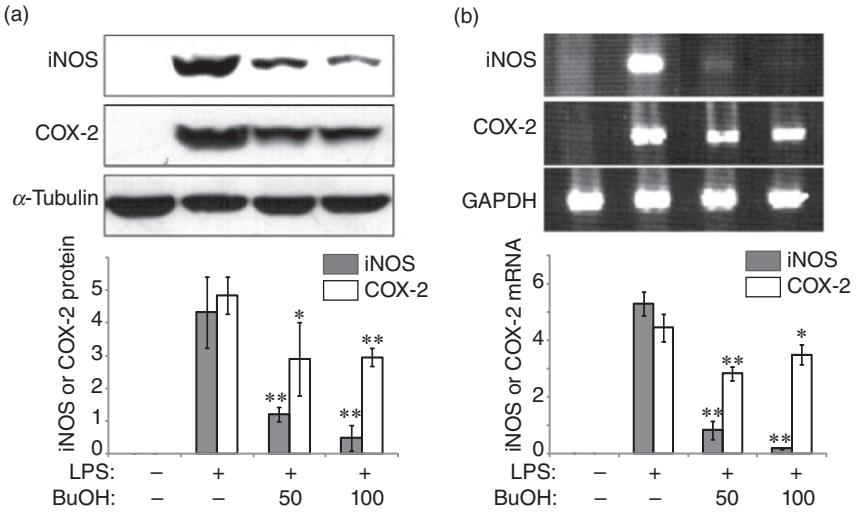

Figure 2 Effects of BuOH extract from RVS on iNOS and COX-2 protein and mRNA expression in LPS-induced macrophage RAW 264.7 cells. Expression levels of protein (a) and mRNA (b). α-tubulin and GAPDH are included as housekeeping controls. Significant difference ($^*P < 0.05$, $^{**}P < 0.01$, $n = 3$) is compared to LPS-treated control.

BuOH extract from RVS exerted the most significant inhibition of NO and ROS production, followed by EtOAc, Et_2O, water and $CHCl_3$ fractions (Figure 1b and 1c). The BuOH extract was therefore used in subsequent experiments designed to determine the anti-inflammatory effects.

Inhibition of iNOS expression by n-butanol extract

To assess whether or not the BuOH extract decreases iNOS and COX-2 levels, the effect of the extract in activated macrophages by LPS was measured. A significant decrease in the iNOS expression was observed after treatment with BuOH extract for 24 h, as observed in the immunoblot analysis (Figure 2a) and the RT-PCR analyses (Figure 2b). However, although COX-2 is also a well-known pharmacological target in relieving inflammation, no dramatic change was shown even at a high concentration of 100 μg/ml of BuOH extract (Figure 2), Nevertheless, the protein and mRNA expression of COX-2 were shown to be inhibited – not dramatically but significantly – by BuOH extract.

Effect of n-butanol extract on Akt and MAPKs signaling

This study also examined, using immunoblotting, the phosphorylation of Akt in LPS-induced macrophage RAW 264.7 cells treated with BuOH extract. After treatment with BuOH extract for 2 h, the expression of total Akt did not change, whereas the basal phosphorylation level of Akt decreased (Figure 3). To further investigate the regulation of MAPKs signaling by the extract, tests were performed to analyse whether BuOH extract prevents the phosphorylation of p38, ERK2 and JNK1 kinases by LPS treatment. The results show that BuOH extract reduces the phosphorylation of ERK2, but there was is significant change in inhibition of p-JNK and p-p38 MAPK activation in LPS-induced macrophages (Figure 3).

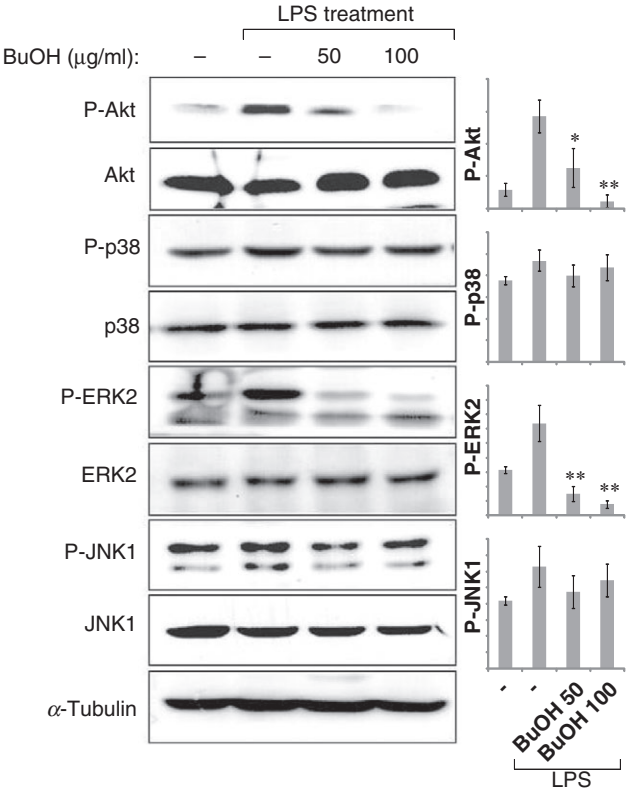

Figure 3 Effects of BuOH extract from *Rhus vernicifera* Stokes on LPS-induced activation of Akt and MAPK kinases. The expression of phospho-ERK1/2 (P-ERK), phospho-p38 (P-p38), phospho-1 JNK1/2 (P-JNK) and phospho-Akt (P-Akt) is analysed by Western blot. Cells were treated with LPS (1 μg/ml) for 30 min. α-tubulin was used as a loading control. Data are obtained from three independent experiments ($^*P < 0.005$, $^{**}P < 0.001$, $n = 3$).

Table 2 Inhibitory effect of BuOH extract from RVS on λ-carrageenan-induced paw edema of rats

Treatment	Increased paw volume (%)					
	1 h	2 h	4 h	5 h	6 h	24 h
Control ($n = 6$)	71.9 ± 23.9	57.6 ± 18.6	54.1 ± 15.5	51.2 ± 13.1	49.7 ± 13.7	50.3 ± 11.3
BuOH ($n = 7$)	63.1 ± 7.4	46.9 ± 10.7	39.7 ± 8.0	36.3 ± 9.7*	35.7 ± 10.4	35.5 ± 11.6*

The amount of BuOH used in this study was 100 mg/kg. Values represent mean ± SD. *$P < 0.05$ is compared with control group.

Table 3 Phenolic compounds from BuOH extract of RVS

Identification	Retention time (min)	Molecular ion	
		$[M + H]^+$	$[M - H]^-$
Fustin[a]	13.9	288.5	286.2
Fisetin[a]	16.9	286.5	N/A
Sulfuretin[a]	18.3	270.6	268.1
Butein[a]	20.7	272.6	270.2

[a]Compared with standards; N/A, not available.

In-vivo effect and standardization of n-butanol extract from RVS

It is also important to know whether or not BuOH extract induces an anti-inflammatory effect in an animal model. We therefore tested if BuOH extract can exert anti-inflammatory effects on carrageenan-induced mouse paw edema. Our results show that treatment with the BuOH extract significantly reduces paw edema volume at 5 and 24 h compared to control ($P < 0.05$, Table 2).

To further evaluate the effective compounds of the BuOH extract from RVS, LC–MS analysis was employed. The BuOH extract contained the four phenolic compounds fustin, fisetin, sulfuretin and butein, based on their respective mass spectra and standards (Table 3 and Figure 4a). The full mass spectrum detected in the UV chromatogram eluting at 14.2 min was additionally analysed. By comparing the spectra achieved at various times of retention, it was found that most of the mass peaks overlaid blank mass peaks, therefore a molecular ion at m/z 287.2198 was considered as a candidate compound (Figure 4b).

To additionally investigate the active compound of BuOH extract, phosphorylations of Akt and ERK in LPS-induced macrophage RAW 264.7 cells treated with fisetin, fustin, butein and sulfuretin were evaluated. Among the four compounds, fisetin was the most effective inhibitor of the phosphorylation of Akt and ERK (Figure 5).

Discussion

Combinations of herbal medicines and Western therapeutics have recently been identified as having pharmacological effects for various diseases. Moreover, since natural products are safer than commercial products, complementary or alternative herbal medicines have been suggested as new targets for drug development.[12–14] In this study, our findings suggest that BuOH extracts of RVS suppress the expression of iNOS via ERK and Akt signaling pathways (Figure 5). Numerous studies have demonstrated that the effectiveness of antioxidants from medicinal plants may be related to their ability to suppress inflammatory responses.[15,16] As reported previously, RVS not only has cytoprotective ability against oxidants but also anti-inflammatory effects on macrophages when they are exposed to LPS. These activities seem to be a result of RVS's phenolic content.[2,17] Although the EtOAc extract had higher phenolic content and DPPH radical-scavenging activity, the BuOH extract exhibited better anti-inflammatory effects than the other extracts. These results suggest that the anti-inflammatory activity of RVS may be correlated more closely with polyphenolic quality than with the content of phenolics. Consistent with this observation, semi-purified BuOH extract displays excellent antioxidant activity and a remarkably potent ability to suppress inflammatory responses.

The macrophage-derived mediators NO and ROS, which were used in the present study, are considered to play a key role in inflammatory and immune responses based on their occurrence at inflammatory sites and their ability to induce many of the hallmarks of the inflammatory response.[18,19] LPS-induced macrophages transiently enhanced the ROS level, and then dropped and immediately returned to the normal level.[20] Furthermore, ROS can activate diverse downstream signaling pathways, such as MAPKs or the nuclear factor, NF-kappa B (NF-κB), thus modulating a number of different steps in the inflammatory cascade, i.e. COX-2 and inducible iNOS.[21–23]

Knowing that iNOS and COX-2 are regulated by upstream signals of Akt and MAPKs such as ERK, c-Jun NH2-terminal kinase (JNK) and p38 MAPK, and that the phosphorylation of the proteins induces NF-κB activation through IκB degradation,[24–27] we tested the effects of BuOH in phosphorylation of Akt and MAPKs. The Akt and MAPKs have been shown to play critical roles in the inflammatory events, leading to lymphocyte activation and production of inflammatory mediators.[28] The BuOH suppressed the phosphorylations of Akt and ERK. These results suggest that inhibition of the signals by BuOH extract might subsequently decrease the expression of iNOS and COX-2.

The compounds of BuOH extract of RVS contained butein, sulfuretin, fisetin and fustin (Table 3 and Figure 4). These compounds may contribute to the inhibition of the inflammatory response.[4,29] Butein suppresses the activation of NF-κB and the secretion of pro-inflammatory cytokines through MAPK signaling.[30,31] In addition, fisetin was shown to exert anti-inflammatory effects *in vivo* and *in vitro*.[4,32] Although this study requires further testing for active compounds depending on collection time and additional quality control of BuOH extraction, fisetin showed the most suppressive effect on the phosphorylation of Akt among the four phenolic compounds, suggesting that fisetin might

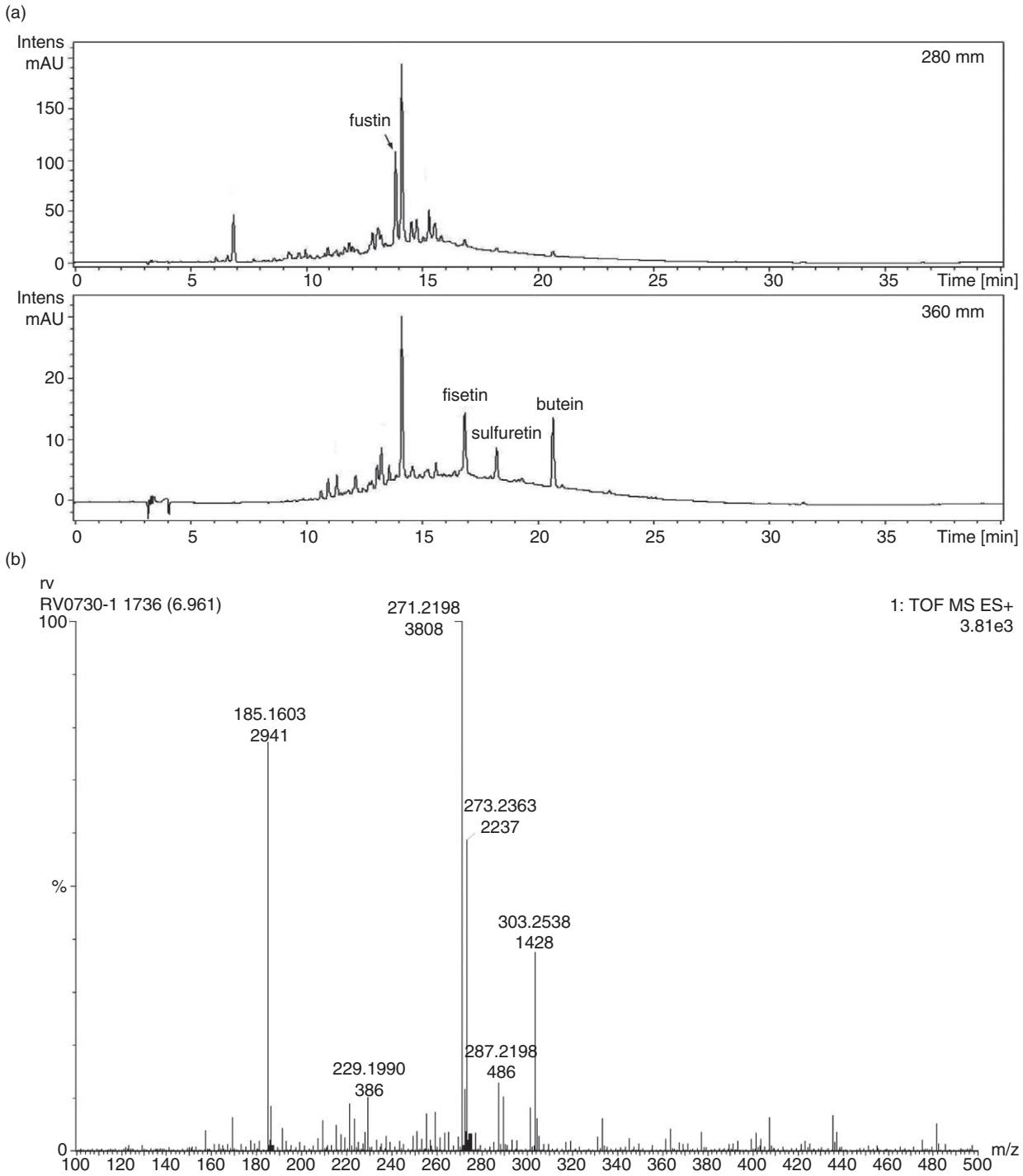

Figure 4 LC-MS chromatogram. (a) BuOH extract from *Rhus vernicifera* Stokes. Peak assignments are shown in Table 3 (b) Mass spectrum of peak at 14.2 min.

be an active compound in the anti-inflammatory effect of the BuOH extract from RVS. Our results suggest that BuOH extract is indeed capable of disrupting the key signal transduction pathways elicited by LPS, subsequently preventing the production of pro-inflammatory mediators, therefore RVS may be a potent agent for inflammation prevention.

Our in-vivo study showed that the BuOH extract of RVS significantly ameliorates mice paw edema, indicating that RVS might have a remarkable ability to suppress inflammatory responses. Butein, also known as 3,4,2′,4′-tetrahydroxychalcone, and a major compound of RVS, reduces carrageenan-induced rat paw edema.[29] The anti-edema activity of the BuOH extract may therefore be evidence

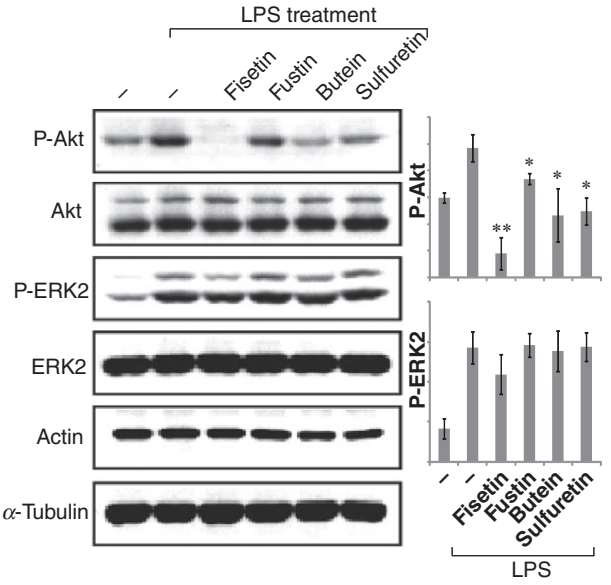

Figure 5 Effects of four phenolic compounds from BuOH extract on LPS-induced activation of Akt and ERK. The expression of phospho-Akt (p-Akt) and phospho-ERK1/2 (p-ERK) is analysed by Western blot. Cells were treated with LPS (1 μg/ml) for 30 min. Actin and α-tubulin are used as a loading control. Fi, fisetin (10 μg/ml); Fu, fustin (150 μM); B, butein (50 μM); S, sulfuretin (50 μM). Data are obtained from three independent experiments (*$P < 0.01$, **$P < 0.001$, $n = 3$).

of the antioxidant activities of RVS, which is possibly responsible for its attenuating inflammatory action.

Since COX-2 is a common molecule in various types of inflammation, including carrageenan-induced paw edema, the activity change of COX-2 may provide an important role of RVS in anti-inflammatory reactions. Further studies on the activities of NOS, COX-2 and related kinases are needed to clarify the anti-inflammatory effect of BuOH extract from RVS. In addition, due to the regulation of iNOS by NF-κB, further analysis of the RVS-induced changes in NF-κB activity may provide a possible mechanism by which RVS-induced suppressions of Akt and ERK could be modulated through NF-κB signaling.

Conclusions

In line with previous findings of the ani-inflammatory effects of RVS, this study confirmed that butein and fisetin identified in the BuOH extract were responsible for suppression of the inflammation-related signals. Although further studies are needed, our findings suggest that RVS could be a candidate agent for prevention of inflammation.

Declarations

Conflict of interest

All authors declare that they have no conflicts of interest to disclose.

Acknowledgements

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