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Full paper

# Gomisin N ameliorates lipopolysaccharide-induced depressive-like behaviors by attenuating inflammation in the hypothalamic paraventricular nucleus and central nucleus of the amygdala in mice

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ABSTRACT

Emotional impairments such as depressive symptoms often develop in patients with sustained and systemic immune activation. The objective of this study is to investigate the effect of gomisin N, a dibenzocyclooctadiene lignan isolated from the dried fruits of *Schisandra chinensis* (*Turcz.*) *Baill.*, which exhibited inhibitory effects of the bacterial endotoxin lipopolysaccharide (LPS)-induced NO production in a screening assay, on inflammation-induced depressive symptoms. We examined the effects of gomisin N on inflammation induced by LPS in murine microglial BV-2 cells and on LPS-induced behavioral changes in mice. Gomisin N inhibited LPS-induced expression of mRNAs for inflammation-related genes (inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, interleukin (IL)-1β, IL-6 and tumor necrosis factor (TNF)-α) in BV-2 cells. Administration of gomisin N attenuated LPS-induced expression of mRNAs for inflammation-related genes, increases in the number of c-Fos immunopositive cells in the hypothalamus and amygdala, depressive-like behavior in the forced swim test and exploratory behavior deficits 24 h after LPS administration in mice. These results suggest that gomisin N might ameliorate LPS-induced depressive-like behaviors through inhibition of inflammatory responses and neural activation in the hypothalamus and amygdala.



## 1. Introduction

Many researchers have previously pointed out that the central nervous system is closely related to the immune system (1,2). Increased pro-inflammatory cytokines cause a coordinated set of adaptive psychological changes such as depression and anhedonia, which are recognized as emotional aspects of sickness behavior, in patients with infection and cancer (1,3,4). These depressive symptoms also occur as a comorbidity of a wide range of illnesses with sustained and systemic inflammation, including stroke, type 2 diabetes, coronary heart disease and rheumatoid arthritis (5–8). Behavioral effects have been well-studied in animal models of inflammation induced by the bacterial endotoxin, lipopolysaccharide (LPS) (9,10). Peripheral administration of LPS induces systemic immune activation, including production of pro-inflammatory cytokines, and causes increased immobility in the forced swim test and decreased exploratory behavior in rodents (11,12). Although how systemic inflammation leads to behavioral changes is not well understood, neural activation in the paraventricular nucleus (PVN) of the hypothalamus and central nucleus of the amygdala (CeA) is likely to be associated with these changes in LPS-treated mice (9,13,14). These studies suggest that regulation of immune and neural activation in the central nervous system (CNS) can ameliorate inflammation-induced behavioral changes. Several studies have shown candidates for treatment of inflammation-induced behavioral changes, such as probiotics (15), agmatine (16), zinc (17) and a hydroethanolic extract of flowers of Pyrostegia venusta, a plant used as a Brazilian traditional medicine (18). We have also reported Japanese traditional medicines as a potential treatment for inflammation-induced depressive-like behaviors (13,19,20).

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Gomisin N, also known as (−)-schisandrin B, is a lignan isolated from the dried fruit of *Schisandra chinensis* (*Turcz.*) *Baill.*, (Schizandrae Fructus) which is used in Japanese Kampo medicines. We previously screened medicinal herb-derived compounds using an *in vitro* assay in murine microglial BV-2 cells, with the goal of finding natural compounds that have ameliorative effects on inflammation-induced depressive-like behaviors. In the assay, we found that gomisin N exhibited the inhibitory effect of LPS-induced nitric oxide (NO) production (our unpublished data). Previous studies have reported various pharmacological activities of schisandrin B such as antioxidant and protective effects against tissue injury of heart, liver, kidney and brain (21–28). These effects led us to speculate that gomisin N may also suppress inflammation in the CNS and ameliorate inflammation-induced behavioral changes. Therefore, in this study, we investigated the effects of gomisin N on LPS-induced inflammation and depressive-like behaviors such as increased immobility in the forced swim test and exploratory behavior deficit in mice.

## 2. Materials and methods

### 2.1. Cell culture

Murine microglial BV-2 cells were a generous gift from Dr. E. Blasi. Cell culture was performed as previously described (13). Briefly, the cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum (Gibco-BRL, Gaithersburg, MD, USA) containing 100 μg/mL streptomycin and 100 IU/mL penicillin in a humidified atmosphere of 95% air/5% $CO_2$ at 37 °C. The cells were plated at a density of $7.5 \times 10^4$ cells/well in 96-well tissue culture plates for Griess and MTS assays, or at $1.0 \times 10^6$ cells/well in a 60-mm tissue culture dish for real-time PCR. Gomisin N was added 1 h before exposure to 0.1 μg/mL LPS.

### 2.2. Animals

Experimental procedures concerning the use of animals were conducted according to the Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society and the committee for Ethical Use of Experimental Animals at Setsunan University. Every effort was made to minimize animal suffering and to reduce the number of animals used. Seven-week-old male ddY mice were obtained from Shimizu Laboratory Supplies Co., Ltd. (Kyoto, Japan) and housed in cages (24 × 17 × 12 cm) in groups of 5 animals under controlled environmental conditions (23 ± 1 °C; 12:12-h light–dark cycle, humidity of 55%, food and water ad libitum) for 1 week before use in experiments. We used 168 mice in total and in single use for each purpose.

### 2.3. Drug preparation and treatment

Dried fruits of *S. chinensis* (3.0 kg; Lot No. 003913003) cultivated in Liaoning province, China were purchased from Tochimoto Tenkaido Co. Ltd (Osaka, Japan) in March 2014, and ground and extracted three times with *n*-hexane. Evaporation of the solvent under reduced pressure gave an *n*-hexane extract (360.0 g), which was chromatographed on silica gel (eluted successively with n-hexane-EtOAc (10:1, 5:1, 2:1, 1:1, v/v) and EtOAc) to give 12 fractions. Fraction 6 (12.4 g) was recrystallized from *n*-hexane-ether to give gomisin N (501.0 mg): colorless prisms (*n*-hexane: ether), HR EI MS *m*/*z*: 400.1888 (calcd for $C_{23}H_{28}O_6$: 400.1886 [$M^+$]), CD ($6.00 \times 10^{-5}$ mol/L, MeOH) Δε (λ nm): −11.15 (252), −9.79 (241), 8.73 (220), $^1$H-NMR (600 MHz, $CDCl_3$) δ: 6.53 (1H, s, H-4), 6.46 (1H, s, H-11), 5.92 (2H, dd, $J$ = 3.8, 1.5, $OCH_2O$), 3.87 (3H, s, 2-$OCH_3$), 3.86 (3H, s, 3-$OCH_3$), 3.80 (3H, s, 14-$OCH_3$), 3.52 (3H, s, 1-$OCH_3$), 2.55 (1H, dd, $J$ = 13.6, 7.3, 6α), 2.50 (1H, dd, $J$ = 13.6, 2.0, H-6β), 2.20 (1H, dd, $J$ = 13.3, 9.5, H-9α), 2.00 (1H, d, $J$ = 13.3, H-9β), 1.87 (1H, m, H-7), 1.76 (1H, m, H-8), 0.95 (3H, d, $J$ = 7.3, 8-$CH_3$), 0.71 (3H, d, $J$ = 7.1, 7-$CH_3$), $^{13}$C-NMR (150 MHz, $CDCl_3$) δ: 151.6 (C-1), 151.5 (C-3), 148.6 (C-12), 141.1 (C-14), 140.0 (C-2), 137.8 (C-10), 134.5 (C-13), 134.1 (C-5), 123.3 (C-16), 121.3 (C-15), 110.6 (C-4), 102.9 (C-11), 100.7 ($OCH_2O$), 61.0 (2-$OCH_3$), 60.5 (1-$OCH_3$), 59.6 (14-$OCH_3$), 55.9 (3-$OCH_3$), 40.7 (C-8), 39.1 (C-6). 35.5 (C-9), 33.5 (C-7), 21.5 (8-$CH_3$), 12.8 (7-$CH_3$) (Supplementary Figs. 1 and 2). $^1$H- and $^{13}$C-NMR spectra were measured on a JEOL JNM-ECA 600 spectrometer ($^1$H at 600 MHz and $^{13}$C at 150 MHz). Chemical shifts are given in δ values (ppm) relative to tetramethylsilane (TMS) as internal standard. EI- and HR-EI-MS spectra (at 30 eV) were obtained by JEOL JMS-700T spectrometer. CD spectra were measured on JASCO J-805 spectropolarimeter. For column chromatography, silica gel 60 (230–400 mesh; Merck, Darmstadt, Germany) was used. Kiesel gel 60 $F_{254}$ (Merck) and RP-18 $F_{254}$ (Merck) were used for analytical TLC. The structure was identified by spectroscopic analysis and comparison with authentic data (29) (Fig. 1). Gomisin N was dissolved in 100% methanol for *in vitro* assays or suspended in 0.5% w/v carboxymethylcellulose (CMC) for *in vivo* tests. LPS (from *Escherichia coli* O127:B8, Sigma, St. Louis, MO, USA) was dissolved in phosphate-buffered saline (PBS) for *in vitro* assays or in saline (0.9% w/v solution of NaCl) for *in vivo* tests. Cells were treated with LPS (0.1 μg/mL) 6 h before *in vitro* mRNA measurement or 24 h before Griess and MTS assays. In mice, LPS (500 μg/kg) was injected intraperitoneally 2 h before decapitation for mRNA measurement or 24 h before behavioral tests or decapitation for immunohistochemistry. Gomisin N was added to cells or injected orally 1 h before LPS treatment. All drugs were injected at a fixed volume of 10 ml/kg body weight.

### 2.4. Griess assay

Griess assay was performed as previously described (13). Briefly, cell-free supernatants were collected and mixed with an equal volume of Griess reagent. Absorbance was measured at 570 nm with a microplate reader (Bio-Rad, Hercules, CA, USA).

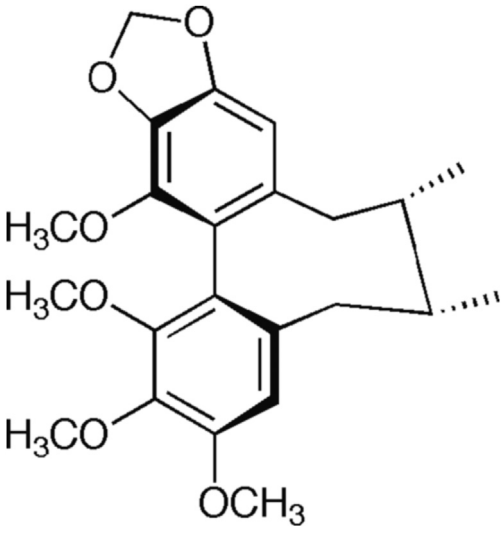


**Fig. 1.** Chemical structure of gomisin N.

### 2.5. MTS assay

MTS assay was performed as previously described (13). Briefly, cell toxicity was measured with a CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI, USA). Absorbance was measured at 490 nm with a microplate reader (Bio-Rad, Hercules, CA, USA).

### 2.6. Quantitative real-time PCR

Total RNA isolated, reverse transcription and quantitative real-time PCR were performed as previously described with the primers indicated in Table 1 (13). Changes in gene expression were calculated relative to the endogenous β-actin standard.

### 2.7. Object exploration test

The object exploration test was performed as previously described (19). Briefly, each mouse was first placed in an observation cage (24 × 17 × 12 cm) and allowed 15 min of habituation. After the habituation period, a novel object (a wooden ball of diameter 5 cm) was placed in the center of the cage and behaviors were videotaped for 5 min. The duration of object exploratory behavior (sniffing or licking the wooden ball) was measured by an observer blinded to the treatment conditions.

### 2.8. Forced swim test

The forced swim test was performed as previously described (30). Briefly, mice were individually placed in a polymethylpentene beaker (height 27 cm, diameter 18 cm) containing 25 ± 1 °C water of depth 13 cm. The performance of the mice for 6 min in the swimming session was videotaped. After the session, mice were removed from the beakers, dried with paper towels and returned to their home cages. The total duration of immobility was measured in the final 4 min of the 6-min test session by an observer blinded to the treatment conditions.

### 2.9. Locomotor activity

Measurement of locomotor activity was performed as previously described (30). Briefly, each mouse was placed individually in a novel cage (30 × 30 × 30 $cm^3$) and locomotor activity was measured using ANY-maze video tracking software (Stoelting Company, Wood Dale, IL, USA).

### 2.10. c-Fos immunohistochemistry

The c-Fos immunohistochemistry was performed as previously described (13). Briefly, mice were deeply anesthetized with pentobarbital and perfused transcardially with saline, followed by a solution of 4% paraformaldehyde. The brain was fixed with 4% paraformaldehyde over 2 days. Serial 50-μm thick coronal sections containing the PVN of the hypothalamus (−0.8 to −1.0 mm with respect to the bregma) and the CeA (−1.0 to −1.2 mm with respect to the bregma) were cut using a microslicer (DTK-1000, Dosaka EM Co., Ltd., Kyoto, Japan). The free-floating sections were incubated in 0.3% hydrogen peroxide, 1% bovine serum albumin containing 0.3% Triton-X, anti-c-Fos rabbit polyclonal primary antibody solution (1:20,000 dilution; Calbiochem, San Diego, CA, USA), biotinylated anti-rabbit IgG (1:200 dilution; Vector Laboratories, Burlingame, CA, USA) and avidin-biotin-horseradish peroxidase complex (Vectastain ABC kit; Vector Laboratories). Brown cytosolic products were obtained by reaction with 3,3′-diaminobenzidine (Sigma, St. Louis, MO, USA). Three independent sections per animal containing the PVN and CeA were selected. c-Fos-positive nuclei were counted manually under bright-field illumination using a microscope (IX71, Olympus, Tokyo, Japan) with a CCD camera (VB-7010, Keyence, Osaka, Japan) by an observer blinded to the treatment conditions. The number of c-Fos-positive nuclei in each section was determined in a 500 × 500 $mm^2$ area in the left and right hemispheres. The mean of this average across three sections was then calculated for each mouse.

**Table 1**
List of primer sequences used in quantitative real-time PCR.

| mRNA | Forward primer sequence (5′ to 3′) | Reverse primer sequence (5′ to 3′) |
|---|---|---|
| iNOS | AGACCTCAACAGAGCCCTCA | GGCTGGACTTTTCACTCTGC |
| COX-2 | GGCCATGGAGTGGACTTAAA | GGGATACACCTCTCCACCAA |
| IL-1β | TGTGAAATGCCACCTTTTGA | CAGGTCAAAGGTTTGGAAGC |
| IL-6 | GTTCTCTGGGAAATCGTGGA | TTCTGCAAGTGCATCATCGT |
| TNF-α | ATGGCCTCCCTCTCATCAGT | CACTTGGTGGTTTGCTACGA |
| β-actin | ACCCACACTGTGCCCATCTA | GCCACAGGATTCCATACCCA |

### 2.11. Statistical analysis

All data are expressed as the mean ± standard error of the mean (SEM) and were analyzed using one-way analysis of variance (ANOVA) followed by Tukey–Kramer *post-hoc* test in Statview 5.0J for Apple Macintosh (SAS Institute Inc., Cary, NC, USA). A value of $p < 0.05$ was considered to be significant.

## 3. Results

### 3.1. Effects of gomisin N on LPS-induced inflammation in BV-2 cells

To identify compounds that can suppress inflammation-induced depressive-like behaviors, we screened 109 medicinal herb-derived compounds using an *in vitro* NO production assay in BV-2 cells (data not shown). Among these compounds, gomisin N (1.6–50 μM) significantly suppressed LPS (0.1 μg/ml)-induced NO production ($F_{7, 16} = 66.327$, $P < 0.0001$) (Fig. 2A). Gomisin N (1.6–25 μM) had no effect on cell viability, whereas 50 μM gomisin N showed cytotoxicity ($F_{7, 16} = 8.706$, $P = 0.0002$) (Fig. 2B). Thus, we examined the effect of gomisin N (12.5 and 25 μM) on LPS-induced mRNA expression of inflammation-related genes (inducible nitric oxide synthase (iNOS), cyclooxygenase (COX)-2, IL-1β, IL-6 and TNF-α) in BV-2 cells. LPS-induced expression of mRNA for iNOS ($F_{3, 8} = 44.697$, $P < 0.0001$), COX-2 ($F_{3, 8} = 103.230$, $P < 0.0001$), IL-1β ($F_{3, 8} = 49.738$, $P < 0.0001$), IL-6 ($F_{3, 8} = 40.546$, $P < 0.0001$) and TNF-α ($F_{3, 8} = 40.505$, $P < 0.0001$) was significantly suppressed by 25 μM gomisin N. In contrast, 12.5 μM gomisin N suppressed LPS-induced expression of mRNA for TNF-α, but not for iNOS, COX-2, IL-1β or IL-6 (Fig. 3A–E).

### 3.2. Effects of gomisin N on LPS-induced inflammation in the hypothalamus and amygdala

Administration of LPS (500 μg/kg) increased iNOS, COX-2, IL-1β, IL-6 and TNF-α mRNA levels in the hypothalamus and amygdala at 2 h after administration. The mRNA levels returned to normal levels at 24 h after administration (data not shown). Gomisin N (100 mg/kg) attenuated LPS-induced mRNA expression in the hypothalamus ($F_{2, 28} = 7.516$, $P = 0.0024$ for iNOS; $F_{2, 28} = 6.706$, $P = 0.0042$ for COX-2; $F_{2, 28} = 8.409$, $P = 0.0014$ for IL-1β; $F_{2, 28} = 9.449$, $P = 0.0007$ for IL-6; $F_{2, 28} = 7.003$, $P = 0.0034$ for TNF-α) and amygdala ($F_{2, 28} = 7.655$, $P = 0.0022$ for iNOS; $F_{2, 28} = 15.232$, $P < 0.0001$ for COX-2; $F_{2, 28} = 16.800$, $P < 0.0001$ for IL-1β; $F_{2, 28} = 20.205$, $P < 0.0001$ for IL-6; $F_{2, 28} = 12.312$, $P = 0.0001$ for TNF-α) (Fig. 4).

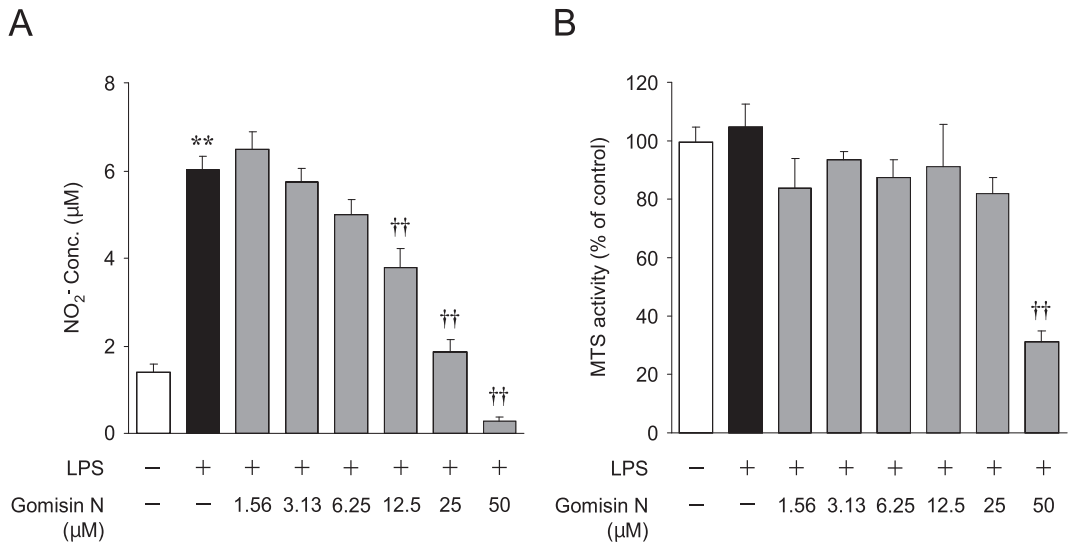


**Fig. 2.** Effects of gomisin N on LPS-induced NO production and cell viability in BV-2 cells. BV-2 cells were treated with gomisin N (12.5–50 μM) and 0.1 μg/ml LPS. (A) The nitrite level was determined by Griess assay. (B) Cell viability was determined by MTS assay. Values are expressed as the mean ± S.E.M. of 3 wells. $^{**}P < 0.01$ vs. control cells. $^{††}P < 0.01$ vs. LPS-treated cells.

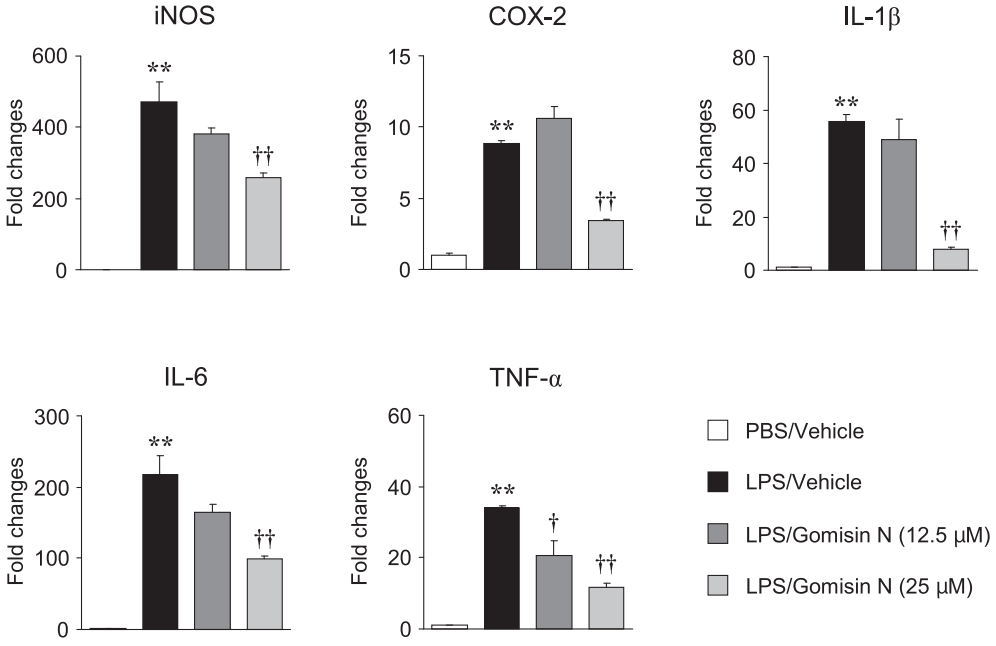


**Fig. 3.** Effects of gomisin N on LPS-induced expression of mRNAs for inflammation-related genes in BV-2 cells. BV-2 cells were treated with gomisin N (12.5 or 25 μM) and LPS (0.1 μg/ml) was added to cultures. The mRNA levels for iNOS, COX-2, IL-1β, IL-6 and TNF-α are shown as fold-changes relative to levels in control cultures. Values are expressed as the mean ± S.E.M. of 3 cultures. $^{**}P < 0.01$ vs. control culture. $^{†}P < 0.05$, $^{††}P < 0.01$ vs. LPS-treated culture.

### *3.3. Effects of gomisin N on LPS-induced neural activation in the paraventricular nucleus of the hypothalamus (PVN) and the central nucleus of the amygdala (CeA)*

Neural activity in the PVN and CeA is persistently increased for at least 24 h after LPS administration, and this persistent neural activation is thought to be associated with behavioral changes such as depressive-like behavior and loss of interest (9,13,14). Thus, to investigate the effects of gomisin N on LPS-induced neural activation, the number of c-Fos immunopositive cells was examined as an indirect neural activity marker (Dragunow and Faull, 1989) in the PVN and CeA at 24 h after LPS administration. LPS-induced significant increases in c-Fos-positive cells in the PVN and CeA, and Gomisin N (100 mg/kg) attenuated these increases in the PVN (Fig. 5A; $F_{2,21} = 18.893$, $P < 0.0001$) and CeA (Fig. 5B; $F_{2,21} = 10.198$, $P = 0.0008$).

### *3.4. Effects of gomisin N on LPS-induced persistent behavioral changes*

LPS induces depressive-like behavior characterized by an increased immobility time in the forced swim test and loss of interest characterized by a decreased time spent exploring a novel object in the object exploration test (13,14). Therefore, the effects of

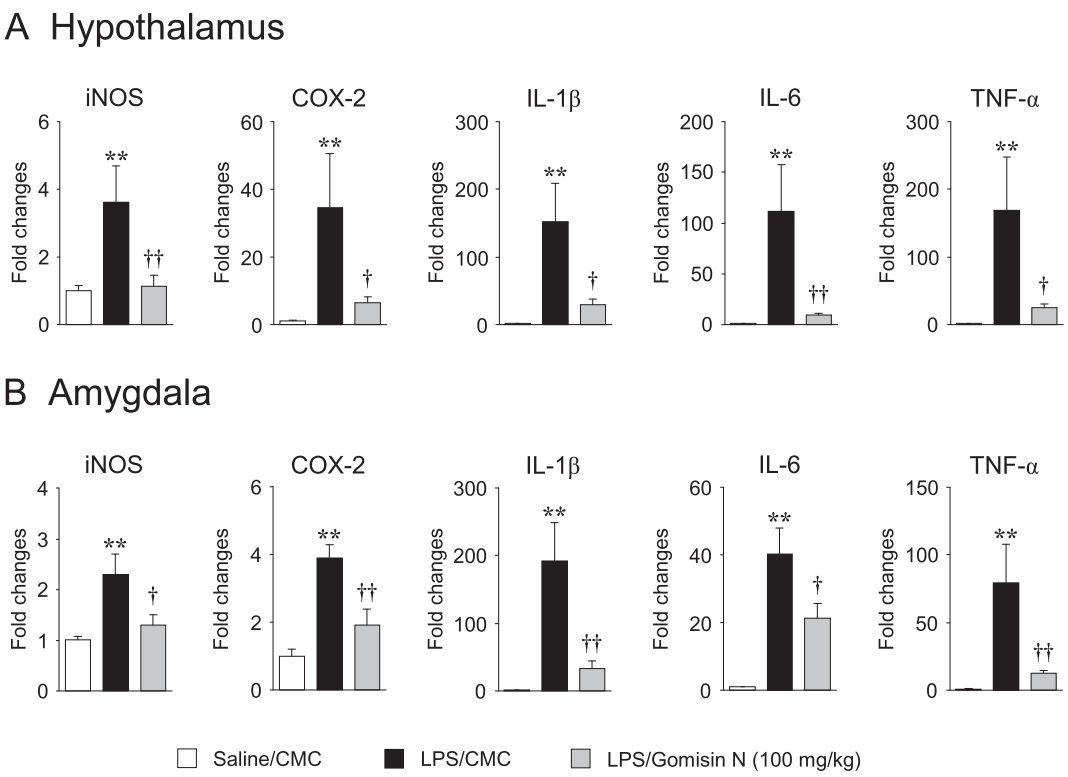


**Fig. 4.** Effects of gomisin N on LPS-induced increases in levels of mRNAs for inflammation-related genes in the hypothalamus and amygdala. The mRNA levels for iNOS, COX-2, IL-1β IL-6 and TNF-α in the hypothalamus (A) and amygdala (B) are shown as fold-changes relative to levels in saline/CMC-treated mice. Values are expressed as the mean ± S.E.M. of 7–12 mice. $^{**}P < 0.01$ vs. saline/CMC-treated mice. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs. LPS/CMC-treated mice.

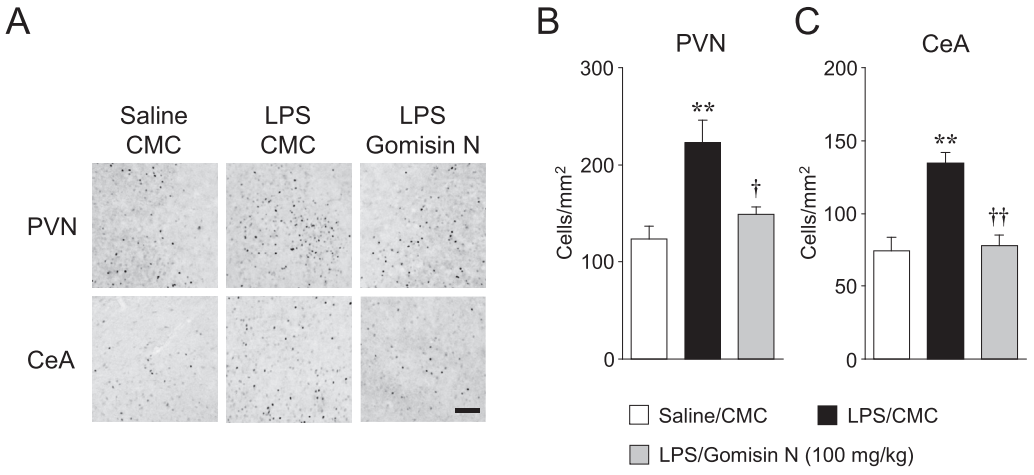


**Fig. 5.** Effects of gomisin N on LPS-induced neural activation in the PVN and CeA. Representative photomicrographs showing c-Fos staining in the PVN of the hypothalamus (top panels) and CeA (bottom panels) and in brain sections of mice treated with saline/CMC (left panels), LPS/CMC (center panels) and LPS/gomisin N (right panels). Scale bars, 100 μm (A). The number of c-Fos-positive cells in the PVN (B) and CeA (C). Values are expressed as the mean ± S.E.M. of eight mice. $^{**}P < 0.01$ vs. saline/CMC-treated mice. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs. LPS/CMC-treated mice.

gomisin N on LPS-induced behavioral changes were examined in these tests. Depressive-like behavior and loss of interest were present at 24 h after LPS (500 μg/kg) administration. Gomisin N (100 mg/kg) significantly reversed the increase in immobility time in the forced swim test (Fig. 6A; $F_{2, 33} = 8.987$, $P = 0.0008$) and the decrease in exploratory behavior in the object exploration test (Fig. 6B; $F_{2, 22} = 10.180$, $P = 0.0007$). LPS and gomisin N did not affect locomotor activity at 24 h after LPS administration (Fig. 6C; $F_{2, 24} = 0.186$, $P = 0.8315$).

## 4. Discussion

Schizandrae Fructus contains several pharmacologically active lignans. In this study, we screened 109 medicinal herb-derived compounds including 3 lignans isolated from Schizandrae Fructus: gomisin N, gomisin A and schisandrin, for effects on LPS-induced NO production. Gomisin N attenuated LPS-induced NO production in a dose-dependent manner. In contrast, gomisin A or schisandrin did not affect LPS-induced NO production (data not

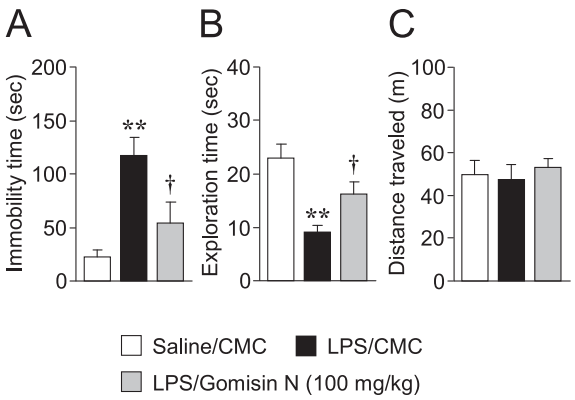


**Fig. 6.** Effects of gomisin N on LPS-induced behavioral changes. (A) Effect of gomisin N on LPS-induced immobility in the forced swim test. (B) Effect of gomisin N on LPS-induced exploratory behavior deficits. (C) Effect of LPS and gomisin N on spontaneous locomotor activity. Values are expressed as the mean ± S.E.M. of 11–13 mice for the forced swim test, 5–10 mice for the object exploration test, and 8–9 mice for analysis of spontaneous locomotor activity. $^{**}P < 0.01$ vs. saline/CMC-treated mice. $^{\dagger}P < 0.05$ vs. LPS/CMC-treated mice.

shown). In a study of structure-activity relationships of dibenzocyclooctadiene lignans isolated from *S. chinensis*, Hu et al (31) found that lignans with a *S*-biphenyl configuration, including gomisin N (Fig. 1), had more potent inhibitory activities on LPS-induced NO production compared to those with an *R*-biphenyl, including gomisin A and schisandrin. These results suggest that the axial chirality of biphenyl is associated with the differences in pharmacological effects on immune activation by lignans.

Inflammation-induced depressive symptoms are triggered by soluble mediators produced by innate immune cells at inflammatory sites. In continuous peripheral immune activation, such as that during infections, cancer and autoimmune diseases, innate immune cells produce pro-inflammatory cytokines that act on the CNS. Microglia in the CNS with macrophage-like activity, including phagocytosis and pro-inflammatory cytokine production, interpret and propagate inflammatory signals initiated in peripheral tissue to the brain. Thus, systemic administration of LPS induces expression of mRNAs for inflammation-related genes such as COX-2, IL-1β, IL-6 and TNF-α in the brain. Systemic or central administration of IL-1β or TNF-α induces depressive-like behavior (32), and LPS-induced behavioral change and production of IL-1β and TNF-α are attenuated in IL-6-deficient mice compared with wild-type mice (33). Prostaglandin synthesis through inflammation-induced COX-2 in the brain is also implicated in depressive-like behavior (10). Intravenous administration of LPS or IL-1β induces COX-2 expression in vasculature-associated cells in brain and neural activation in the PVN (34,35). We and others have shown that neural activation in the PVN and CeA plays a role in LPS-induced depressive-like behavior and loss of interest (9,13,14). These behaviors are thought to be caused by pro-inflammatory cytokine-induced neural activation. Moreover, in this study, LPS-induced neural activation was observed at 24 after LPS administration in the PVN and CeA, nevertheless the expression of mRNAs for inflammation-related genes return normal levels (data not shown), which suggests that inflammation might be just a trigger of neural activation and that neural activation, but not pro-inflammatory cytokines, might be directly involved in LPS-induced behavioral changes observed at 24 after LPS administration. Gomisin N inhibited LPS-induced NO production and increased mRNA levels for iNOS, COX-2, IL-1β, IL-6 and TNF-α in murine microglial cell line BV-2 (Figs. 2A and 3). Similar findings for gomisin N have been reported in a murine macrophage-like cell line RAW264.7 (36). Collectively, these results suggest that gomisin N has anti-inflammatory activity in the central and peripheral immune systems, and indicate that gomisin N may have an ameliorative effect on inflammation-induced behavioral changes. This is consistent with our results showing that gomisin N attenuated LPS-induced increases in mRNAs for inflammation-related genes and c-Fos immunopositive cells in the hypothalamus and amygdala, and improved depressive-like behavior and loss of interest (Figs. 4–6).

LPS-induced behavioral changes can be divided into transient changes such as decreases in locomotor activity and food intake, and persistent changes such as depressive-like behavior and exploratory behavior deficit (9,14). In rodents, inoculation of tumor cells induces depressive-like behavior and exploratory behavior deficit, but does not decrease locomotor activity and food intake (19, 37). Haba et al (14) suggested that an exploratory behavior deficit that probably reflects loss of interest is one of the most vulnerable behaviors to the effects of LPS administration. Thus, depressive-like behavior can be regarded as common symptoms of inflammation-induced behavioral changes. We have previously shown that LPS (500 μg/kg, i.p.) induces persistent behavioral changes such as increased immobility in the forced swim test and decreased exploration to a novel object which last even after locomotor activity returned in ddY male mice. To evaluate effects of gomisin N on LPS-induced depressive-like behaviors under normal locomotor activity, thus, we examined behavioral tests 24 h after administration of LPS at 500 μg/kg in this study. We performed the forced swim test and novel object exploration test to investigate the effect of gomisin N on LPS-induced depressive-like behavior and loss of interest. Gomisin N inhibited LPS-induced immobility in the forced swim test and exploratory behavior deficit in the novel object exploration test without affecting locomotor activity. These results suggest that gomisin N has ameliorative effects on the depressive symptoms in patients with sustained and systemic inflammation. Schizandrae Fructus is an ingredient of Ninjinyoeito, a Japanese Kampo medicine, which could be used for the deconditioned patients with a malignant tumor. It can be expected that Kampo medicines containing Schizandrae Fructus may ameliorate depressive symptoms of sickness behavior in tumor patients due to anti-inflammatory effect of contained gomisin N.

In conclusion, this study shows that gomisin N ameliorates LPS-induced depressive-like behavior and loss of interest, which are putative core symptoms in patients with sustained and systemic inflammation. These ameliorative effects of gomisin N are likely to occur through suppression of inflammation and neural activation in the PVN and CeA. These results indicate that Schizandrae Fructus may have potential therapeutic value for treatment of depressive symptoms in patients with sustained and systemic inflammation.

## Conflicts of interest

The authors declare that there are no conflicts of interest regarding publication of this paper.

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## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jphs.2016.09.004.

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